KU Leuven
Group Biomedical Sciences
Faculty of Pharmaceutical Sciences
Laboratory of Medicinal Chemistry

Design and synthesis of new compound libraries targeting kinases

Hassen BEL ABED

Doctoral thesis in Pharmaceutical Sciences

Leuven, 2013

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Acknowledgements

Summary

This thesis was devoted to the synthesis of novel heterocycles and more particularly fused rings in order to develop a number of libraries aiming to be screened towards different kinases incorporated in ongoing projects of Galapagos.

In the first chapter of this PhD research, a brief introduction concerning protein kinases and kinase inhibition is described.

The second chapter deals with the synthesis of a library of pyrazolo[1,5-a]pyrimidine derivatives from the elaboration of the strategy to the biological evaluation. Moreover, the development of a facile approach to 3-fluoro pyrazolo[1,5-a]pyrimidine has been described.

The third chapter is devoted to the synthesis of pyridazines and related fused rings, a convenient methodology to functionnalized pyridazine derivatives has been elaborated allowing the introduction of ester, ketone or sulfonyl groups. The extension of this strategy led to the synthesis of novel fused pyridazines such as 5H,6H,7H-pyridazino[4,3-e][1,4]diazepine. A final part describes the development of the first catalytic Diaza-Wittig reaction and a novel catalytic Aza-Wittig applied to the synthesis of pyridazines and 1,4-benzodiazepine. All the analogues have been screened against a number of kinases.

The fourth chapter discuss about the three different isomers of triazolopyridine, in a first part efforts have been directed towards the synthesis of triazolo[4,3-a]pyridine, two patterns of substitution have been considered but only one has been pursued due to synthetic problems. During the elaboration of the library, the formation of an isomer has been observed and then identified as the triazolo[1,5-a]pyridine obtained by the possible Dimroth rearrangement enhanced by the presence of a nitro group in our starting material. A route has been studied in order to synthesize this isomer. The last isomer explored was the triazolo[4,5-b]pyridine. Three libraries have been developed and screened by Galapagos.

Samenvatting

Dit doctoraatsonderzoek is gewijd aan de synthese van nieuwe heterocyclische verbindingen en meer in het bijzonder gefuseerde ringen dit om verschillende bibliotheken te screenen dit gericht zyn naar verschillende kinases dewelke opgenomen zyn in lopende projecten Galapagos.

In het eerste hoofdstuk van dit promotieonderzoek, een korte inleiding over proteïne kinasen en wordt kinaseremming beschreven.

Het tweede hoofdstuk behandelt de synthese van een bibliotheek van pyrazolo[1,5-a]pyrimidine derivaten en de uitwerking van de strategie voor de biologische evaluatie. Hiervan, Bovendien is de ontwikkeling van een gemakkelijke benadering voor 3-fluor pyrazolo[1,5-a]pyrimidine beschreven.

Het derde hoofdstuk is gewijd aan de synthese van pyridazinen en aanverwante gefuseerde ringen. Een handige methode om gefunctionaliseerd pyridazine derivaten is uitgewerkt, waardoor de introductie van ester, keton of sulfonylgroepen mogelijk. De uitbreiding van deze strategie leidde tot de synthese van nieuwe gecondenseerde pyridazinen zoals 5H, 6H, 7H-pyridazino[4,3-e][1,4]diazepine. Een laatste deel gaat over de ontwikkeling van de eerste katalytische Diaza-Wittig reactive en een nieuwe katalytische Aza-Wittig reactie die toegepast is op de synthese van pyridazines en 1,4-benzodiazepine. Alle analogen zijn gescreend tegen een aantal kinasen.

In het vierde onderdeel behandelen we drie verschillende isomeren van triazolopyridine, in een eerste gedeelte zijn pogingen gericht op de synthese van triazolo[4,3-a]pyridine, twee patronen van substitutie warden beschouwd, maar slechts één is uitgevoerd door synthetische problemen. Bij de uitwerking van de bibliotheek, is de vorming van een isomeer waargenomen en geïdentificeerd als het triazolo[1,5-a]pyridine. Dit isomer werd verkregen door de Dimroth omlegging versterkt door de aanwezigheid van een nitrogroep in ons uitgangsmateriaal. Een route is bestudeerd om deze isomeer synthetiseren. De laatste die isomeer verkend werd was het triazolo[4,5-b]pyridine. Drie bibliotheken werden ontwikkeld en gescreend door Galapagos.

List of abbreviations

***p*-ABSA** *p*-acetamido benzene sulfonyl azide

Ac acetyl

Ac_2O acetic anhydride

ATP adenosine triphosphate

Bn benzyl

Boc *tert* –butyloxycarbonyl

BuLi *n*-butyl lithium

CDK9 cyclin dependent kinase 9

Dabal-Me_3 Bis(trimethylaluminum)–1,4-diazabicyclo[2.2.2]-octane adduct

DABCO 1,4-diazabicyclo[2.2.2]octane

DBU 1,8-Diazabicyclo[5,4,0]undec-7-ene

DIPEA diisopropylethylamine

DMF dimethylformamide

DMF-DMA dimethylformamide-dimethylacetal

DMSO dimethyl sulfoxide

DPPA diphenylphosphorylazide

EDCI *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide

Et ethyl

GABA *g*-aminobutyric acid

GSK3 glycogen synthase kinase 3

HIV human immunodeficiency virus

HMPT hexamethyl phosphorus triamide

IBX 1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide

IC_{50} half maximal (50%) inhibitory concentration

***i*-Pr** isopropyl

***i*-Pr_2O** diisopropyl ether

IRAK4 interleukin-1 receptor-associated kinase 4

JAK Janus kinase

K_3PO_4 potassium phosphate

LiHMDS lithium hexamethyldisilazane

LRRK2 Leucine-rich repeat kinase 2

MAP4K4 mitogen-activated protein 4 kinases 4

Me methyl

NBS *N* –bromosuccinimide

NMP *N*-methyl-2-pyrrolidinone

NOESY nuclear overhauser effect spectroscopy

Nu nucleophile

OEt ethoxy

p38 MAP p38 mitogen activated protein kinase

PBu_3 tributylphosphine

PDE phosphodiesterase

PEt_3 triethylphosphine

Ph phenyl

PIM proviral integration site of moloney murine leukemia virus

PKA protein kinase A

$POCl_3$ phosphorus oxychloride

$P(OEt)_3$ triethylphosphite

PPA polyphosphoric acid

PPh_3 triphenylphosphine

rt room temperature

SN_{Ar} nucleophilic aromatic substitution

TBTU *N,N,N',N'*-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate

TFA trifluoroacetic acid

TGF b II tumor growth factor b II receptor

THF tetrahydrofuran

TLC thin layered chromatography

VEGFR vascular endothelial growth factor receptor

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Chapter I. General introduction

1. Protein kinases

The Phosphorus has been identified as a very important element in cellular metabolism since more than 100 years, the first example has been observed in the fermentation by yeast, in fact the phosphate group was described as a prerequisite.[1] During the following decades, high energy phosphate esters and particularly the adenosine triphosphate known as ATP, have been identified as main sources of energy for cells. The concentration of ATP in cells is known to be high, generally between 1 to 5 mM,[2] and most of the cellular processes and pathways depend on the hydrolysis of ATP which is converted into inorganic phosphate and adenosine diphosphate (ADP).

In 1955, Krebs and Fischer published[3] a study concerning the biochemistry of glycogen phosphorylase, the critical role of this enzyme was the release of glucose in order to provide energy for the cells. At the time of their discovery, this phosphorylase was previously known in two forms, the active glycogen phosphorylase *a* and the inactive glycogen phosphorylase *b*. The study of Fischer and Krebs demonstrated that the conversion from the inactive form to the active form was catalyzed by an enzyme. The phosphorylase kinase was the name chosen for the discovered enzyme, the kinase transferred the γ-phosphate of adenosine triphosphate towards the protein.[4] This report was the first demonstration showing the essential role of kinases in the regulation of biochemical signaling pathways. This discovery was awarded by the Nobel Prize in physiology/medicine in 1992 "for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism".[5]

After the research of Fischer and Krebs, many groups have tried to identified phosphorylation of aminoacid residues different than serine or threonine and in 1980,[6] it was demonstrated that the tyrosine residue was also phosphorylated. Various tyrosine kinases have been identified during the 1980s, it was shown that these kinases can be receptor tyrosine kinases but also non receptor tyrosine kinases. This variety of enzymes was recognized as having a key role in cellular signaling functions but also having an important role in oncology. Due to large amount of kinases (518 kinases), a classification has been established dividing them in two sub-families tyrosine kinases and serine/threonine kinases, themselves sub-divided in other families. Although,

kinases are divided in two main families corresponding to the residue phosphorylated, several examples showed that particular kinases can phosphorylate both classes such as mitogen activated protein kinase kinases (MAPKKs or MAP2Ks), it was observed that phosphorylation was performed with threonine and tyrosine residues.

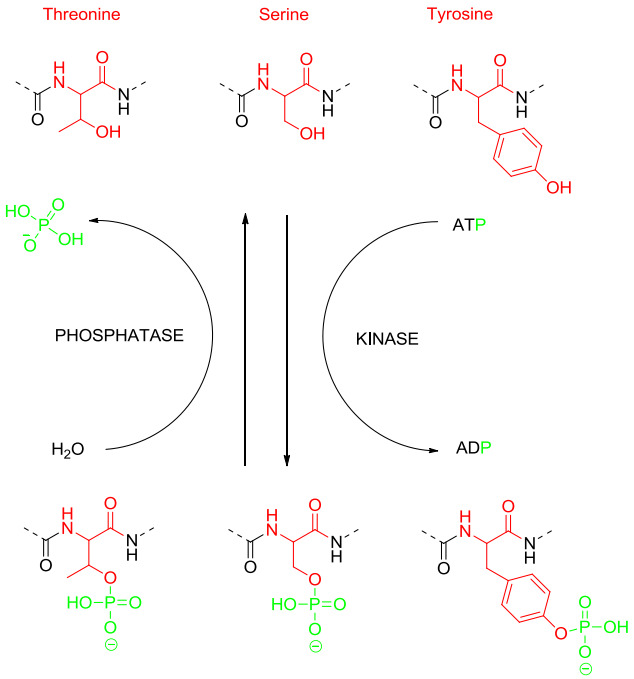

Figure 1. Kinase phosphorylation and phosphatase dephosphorylation.

In mammalian species, it was described that the protein kinases are highly conserved and share also a high degree of homology with other species from the eukaryotic kingdom.[7] A number of studies on eukaryotes led to the identification of twelve subdomains in kinases. Moreover, X-ray crystal structures of protein kinases have shown that these domains are between two major lobes comprising the ATP binding site. These two lobes correspond to the N-terminal lobe constituted by subdomains I-V and the C-terminal lobe comprising the subdomains VI-XI. Each subdomain contains an important feature to make in order the catalytic machinery of the kinase, the role of the subdomain I is to bind the nucleotide with the help of its glycine region. The subdomains I and III interact with the ATP by forming a salt bridge with their respective lysine and glutamate region. The salt bridge formed to interact with the ATP by these 2 subdomains is very important for the regulation of most of the kinases.[8]

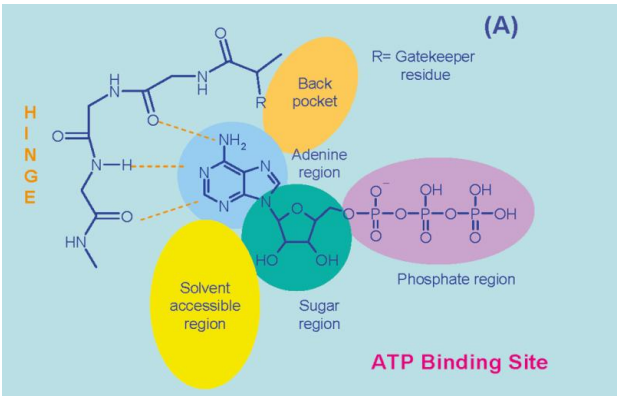

Figure 2. ATP binding site.

All the studies performed during the last decades have demonstrated the importance of the phosphorylation by the kinases. It has been clearly identified as a universal activation mechanism that controls the regulation of most of the cellular processes. The role of the phosphatases[9] has also been described as crucial for such regulation, in fact the dephosphorylation of protein (inverse reaction than kinase) is complementary to the phosphorylation catalyzed by the kinases.

Protein kinases have been classified in different classes.[10] The classification has been based on the specificity of the substrate phosphorylated by the catalytic domains, in fact such classification associates kinases to the amino acids they phosphorylate. Two main classes have been created, the first one phosphorylating tyrosine residue is tyrosine kinases (TKs), and the second one phosphorylating serine or threonine residue is serine-threonine kinases. The structure of these two classes has many homologies such as a glycine-rich region in the N-terminal lobe and an ATP-binding pocket.[11]

The two classes previously discussed are themselves divided in sub-families, the tyrosine kinases for example are divided into two large families, the receptor tyrosine kinases and the non-receptor tyrosine kinases. The receptor class corresponds to the transmembrane proteins with an extracellular domain, which bind the ligands and an intracellular kinase domain, the non-receptor tyrosine kinases do not possess a transmembrane domain and are located in the cytoplasm, the nucleus and the plasma membrane. The catalytic machinery involved for the two sub-classes of tyrosine kinases is highly regulated in order to avoid the uncontrolled proliferation of phosphorylated tyrosine residue. The first class identified was the tyrosine kinases and many studies have led to partial understanding of this family. To date, around 90 tyrosine kinases and 43 tyrosine kinases like have been identified. Amongst the tyrosine kinases, 58 have

been identified to be receptor, the receptor tyrosine kinases are subdivided based on their different properties such as structural organization, 20 subclasses have been numbered. The remaining tyrosine kinases (32) correspond to the non-receptor type and have been themselves classified into 10 subfamilies according to their properties. As previously mentioned, these kinases regulate various cellular processes such as differentiation, survival, cellular proliferation, mobility and functions. A large amount of malignant human cancers are due to uncontrolled receptor tyrosine kinases. Manning et al. was able to classify all the kinases into a human kinome divided into seven families: AGC, CAMK, CK1, CMGC, STE, TK and TKL.[12]

Some of the kinases are receptor types and are located on the cell membrane, other kinases have a role of signal mediator and are found in the cytosol of the cells. The receptor kinases are activated by ligands at the cell surface transmembrane, this activation leads to a cascade of intracellular mechanisms in order to modify the gene transcription and a cell response. The unregulated activity of the kinase can lead to a loss of mediators controlling the inhibition or the formation of mutations which can lead to abnormal cell proliferation, one of the symptoms involved in cancer.[13] In receptor tyrosine kinases, the catalytic domain has been well described and the different processes involved in the phosphorylation such as the ligand binding and the activation of the domain have been also detailed.[14] In most of the cases, a ligand activates the receptor tyrosine kinase by binding to an external region, the activation of the intracellular kinase domain is followed by an autophosphorylation of the residue and the activation of a cellular pathway or phosphorylation cascade.[15]

Two conformations have been put in evidence in receptor tyrosine kinases, the catalytic domain can be active or inactive (open or close form). These two conformations differ by the position of the activation loop. While the conformation is active, the activation loop is turned outside of the ATP binding pocket, allowing the binding of protein residue. In the inactive conformation, the activation loop is blocking the entrance of the ATP binding pocket, in this closed conformation no substrates can be phosphorylated.[16] The inactive state for a receptor tyrosine kinase is obtained while no ligands are bound, the kinase stays in a unphosphorylated state and as a monomer (ligands required to achieve the dimerization step).[17] In contrast to previously mentioned the activation by ligands binding to the extracellular domain of receptor

tyrosine kinases generates the active conformation leading to the receptor homo or heterodimerization followed by autophosphorylation of a tyrosine residue in the activation loop of the catalytic domain.

While the receptor tyrosine kinases are activated, a number of intracellular signalling pathways are launched such as SH2- or SH3-mediated which interact with others proteins located in the cytoplasm, in parallel signaling enzymes such as protein kinase C, phospholipase C, PI3K or mitogen-activated protein kinase are activated.[18] The receptor tyrosine kinases possess an auto-inhibitory domain at the surface of the cell in contrast to the non-receptor tyrosine kinases, which do not have, these kinases are generally in the inactive conformation due to inhibitory proteins performing an auto-inhibition of the kinases. The activation of the non-receptor tyrosine kinases is possible due to intracellular signals which dissociate the inhibitory proteins, by attraction of existing receptors located at the cell transmembrane leading to a dimerization followed by autophosphorylation. The termination of this signaling cascade initiated by the non-receptor tyrosine kinases signalling is observed by the action of tyrosine phosphatases which hydrolyse phosphate groups contained by the tyrosine residues and also by the formation of inhibitory molecules helping to the termination of this process.[19]

The unregulated kinases can lead to aberrations in proliferation, cellular growth and survival pathways, such cases result in the formation and proliferation of tumored cells and generate cancer or diseases associated. Cancer and all related diseases represent one of the most challenging health problems of the 21st century. In 2008, The American Cancer Society has estimated that more than one million cases of cancer were diagnosed in the USA alone and that over half a million people died from their disease.[20] The National Institutes of Health has made an estimation that the additional cost of cancer, including direct medical costs, corresponded to more than $200 billion.

The most common way for cancer to grow is the development of tumored cells in epithelial tissues, which after expansion become a tumor tissue or carcinoma. The other sources for non-epithelial cancers are the hematological malignancies, sarcomas, neutrally derived tumors and melanoma. At the beginning of the formation of a tumor, only signs of health problems and morbidity are observed but the lethality of cancer is generally linked to the invasion by a large amount of tumored cells and metastasis.

When tumored cells become malignant, they can migrate from their initial location of development to local and distal parts in the human in order to proliferate.[21]

Knowing that protein kinases play a critical role in the control of several intracellular signaling pathways, the dysregulation of the kinase activity has been identified as common for most of the cancer and related diseases. This observation led to consider kinase inhibitors with a strong attention as targets for drug discovery projects.[22]

The important attention attached to the development of kinases inhibitors was not only restricted to cancer but also extended towards other indications such as inflammatory diseases. In the last decade, the considerable efforts put in this field have been rewarded by a number of candidates for clinical trials and successful drugs on the market such as Gefitinib.[23] Several kinase inhibitors compiling a large structural variety have been approved for the treatment of tumoral diseases, a large number of putative kinase inhibitors are submitted every year to clinical evaluation. The development of kinase inhibitors by the screening of large library of compounds and the study of structure-activity relationship, in addition to X-ray crystal structures and computational studies, have led to significant improvements in the understanding of the catalytic domain structure. Resulting from this research, the exploration of small molecule as kinase inhibitors has been better directed and the design of selective or potent inhibitors can be allowed. As discussed for the classification of the protein kinases, it is also possible to classify the kinase inhibitors into two classes, the first class corresponding to the inhibitors targeting the active form or "DFG-in" conformation of the kinase (type I inhibitors). The second class corresponds to the inhibitors of the inactive form or "DFG-out" (type II inhibitors). The two classes of kinase inhibitor present advantages and inconvenient but none of them have proved to be a favorised strategy towards the development of a successful inhibitor for clinical trials.

Due to the fact that kinases can be in an active or inactive conformation, many investigations have been performed to enhance the selectivity of the putative kinase inhibitor. It was stated that the possibility to target one of the two state of activation distinctly could lead to the design of kinase inhibitors more specific to a particular kinase. In fact, the active conformation possesses a high structural homology in comparison with the inactive conformation which displays a higher structural diversity.

These investigations led to the conclusion that the aim of selective kinase inhibitors will be achieved by selecting to target the inactive form of the kinase.

The majority of the kinase inhibitors developed to date are ATP-competitive but few notable exceptions are known such as MEK inhibitors[24] which are not competitive with ATP. The first strategy envisioned was to design kinase inhibitors in order to compete ATP because it was believed to be selective, in fact the high structural homology of the ATP binding site in the active conformation led to this misstatement. Moreover, the high concentration of ATP in the cells is known to be an important feature to overcome for ATP-competitive kinase inhibitors. Effective kinase inhibitors will display an important inhibition and potency in order to compete with ATP in catalytic domain.[25] The development of non-competitive kinase inhibitors are favorised due to no effect with the ATP concentration. Despite the fact that ATP concentration could have been a major issue for the development of kinase inhibitors, it was shown that it was not impossible.

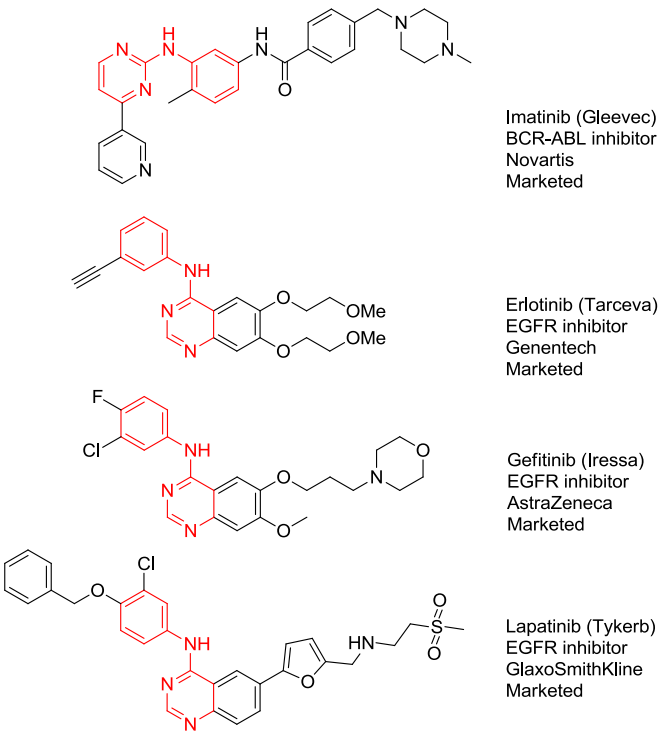

Figure 3. kinase inhibitors.

2. Aim of the thesis

The aim of this PhD was to develop the synthesis of different scaffolds (mainly fused rings) and a library associated to each structure in order to evaluate the biological activity towards kinases. More than ten different scaffolds have been developed during this PhD research but with a view of clarity, only three families of scaffold will be discussed in details during the following chapters.

In the first chapter, the synthesis of a series of compounds based on the pyrazolo[1,5-a]pyrimidine scaffold is discussed, the different routes investigated and the achievements are described, moreover, the development of new 3-fluoro pyrazolo[1,5-a]pyrimidine derivatives are presented. The second chapter describes the development of short and convenient routes for the construction of pyridazine derivatives and the first synthesis of fused pyridazines associated such as dihydro-pyridazino[4,3-e][1,4]diazepine. The third chapter of this study is focused on the synthesis of different triazolopyridine isomers and introduce the Dimroth rearrangement applied to the triazolo[4,3-a]pyridine. All the compounds synthesized during this PhD have been screened against specific kinase targets found in Galapagos projects.

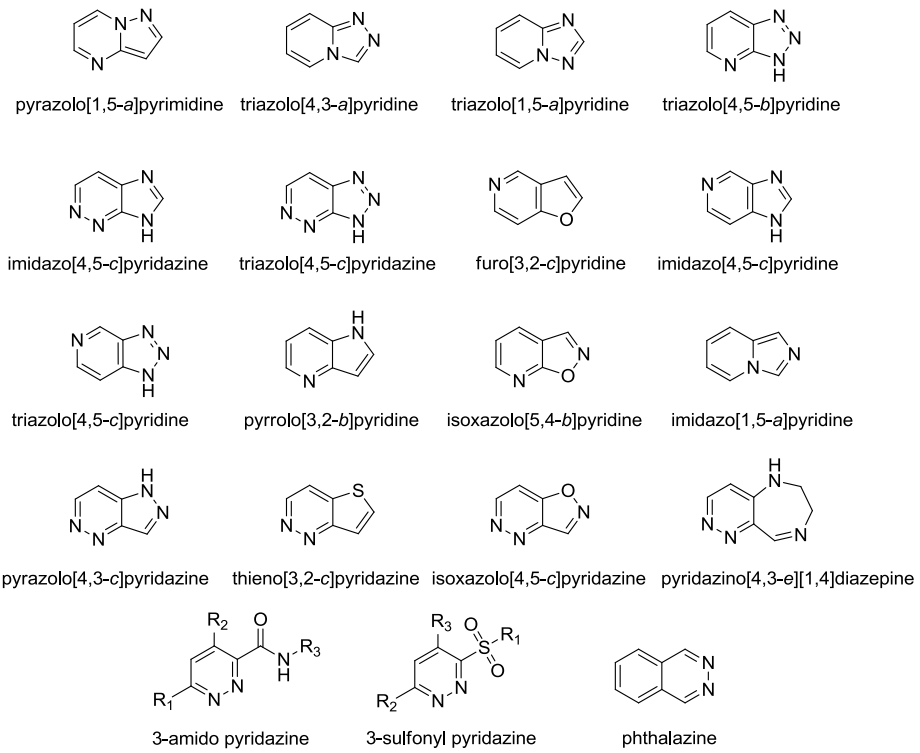

Figure 4. List of the scaffolds synthesized during the PhD.

During the last four years, the construction of 17 fused rings and 2 novel pyridazine derivatives have been developed, most of them led to a library synthesis of more than 250 compounds to date. This report dealt in details mainly with the synthesis of pyrazolo[1,5-a]pyrimidine derivatives, the different triazolopyridine analogues, the simple approaches to novel pyridazines, the first synthesis of fused pyridazine derivatives and the elaboration of the first organophosphorus-catalyzed Diaza-Wittig reaction.

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Chapter II. Synthesis and biological evaluation of a pyrazolo[1,5-a]pyrimidine library

1. Introduction

Over the past few decades, the pyrazolo[1,5-a]pyrimidine has surfaced in a variety of biologically active molecules[1] such as the α1β2γ2-selective ligand **2.1**, the anti-schistosomal agent **2.2**,and the anti-inflammatory **2.3**.

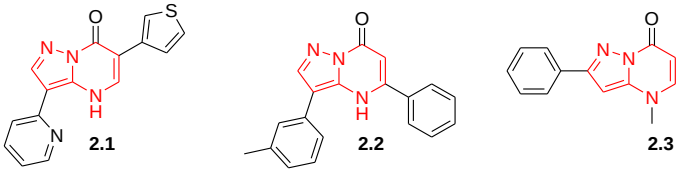


Figure 1. Biologically active pyrazolo[1,5-a]pyrimidine.

Pyrazolo[1,5-a]pyrimidine has been studied in kinase inhibition and several compounds have been described such as Wyeth research potent B-Raf kinase inhibitor **2.4** or a cyclin dependent kinase inhibitor **2.5** developed by Vernalis.[2]

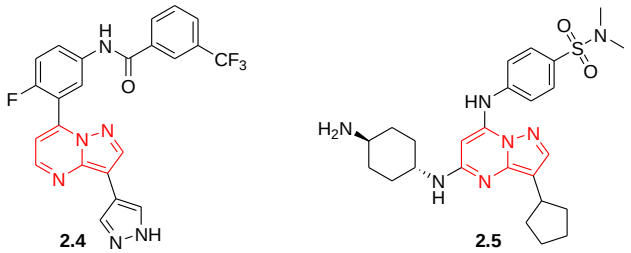


Figure 2. Kinase inhibitors based on pyrazolo[1,5-a]pyrimidine.

This core has shown inhibitory activity towards different kinases like B-Raf, CDK or Pim for example. Selectivity being an important factor in kinase inhibition, the substitution pattern and the kind of decoration used allowed the selectivity towards particular kinases with pyrazolo[1,5-a]pyrimidine that has itself interactions with the hinge region of the ATP binding site.

This scaffold is extensively used and recently Array BioPharma and Genentech have described a novel series of ATP competitive B-Raf inhibitors[3] with excellent cellular potency and selectivity towards the target with an IC_{50} = 43nM. In this study, the pyrazolo[1,5-a]pyrimidine **2.6** has been substituted by different heterocycles and it results with a decrease of the kinase inhibition while using pyridine **2.7**, quinoline **2.8** and especially with the isoster imidazo[1,2-a]pyrimidine **2.9**.

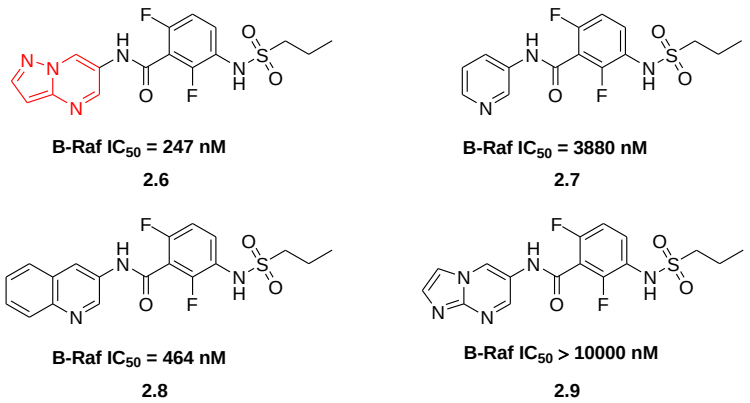


Figure 3. Scaffold substitution of a B-Raf inhibitor.

Teijin Pharma and BioFocus have discovered a novel class of selective mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2) inhibitors[4] using a focused library and structure-based approach to identify a series of pyrazolo[1,5-a]pyrimidine derivatives. It was possible to determine the binding mode of the hit **2.10** by binding the X-ray crystal structure of **2.10** to crystalline MAPKAP-K2, the hinge binding is performed by the scaffold as previously discussed and the selectivity obtained due to the substitutions of the pyrazolo[1,5-a]pyrimidine.

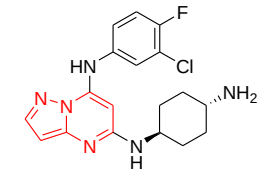


Figure 4. MAPKAP-K2 inhibitor.

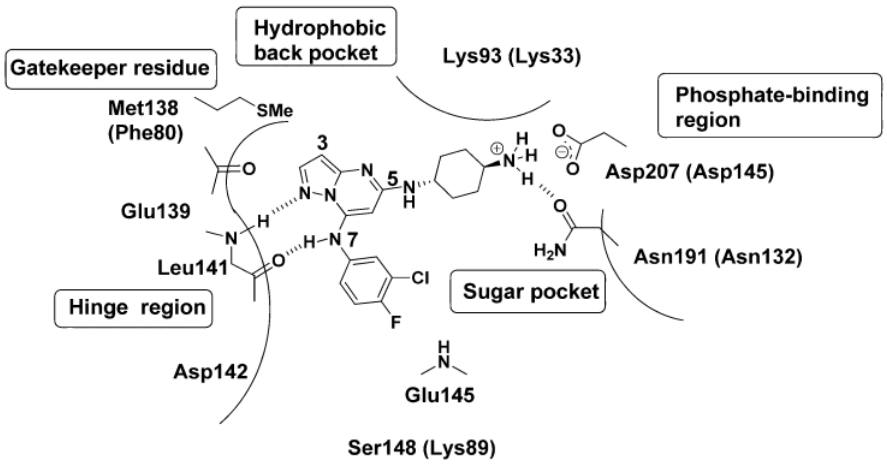
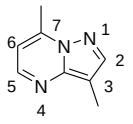


Figure 5. Binding mode of **2.10**.

In view of previous literature describing this structure as potential scaffold to develop kinase inhibitor, the synthesis of a library of pyrazolo[1,5-a]pyrimidine **2.11** was set up in which substituents on the positions 3 and 7 were varied in a systematic way.

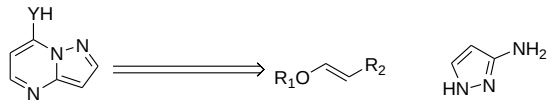


2.11

Figure 6. Scaffold explored **2.11**.

2. Results and discussion

The synthesis of pyrazolo[1,5-a]pyrimidine started with a retrosynthetic study of the scaffold based on literature search. The ideal starting material for the synthesis is the commercially available 3-aminopyrazole **2.12**, in fact it is necessary to use a 1,3-N,N-bis-nucleophile to introduce the 3 nitrogens of the scaffold. One of the method used is the condensation of **2.12** with alkyl alkoxy acrylate or alkoxy acrylonitrile **2.13** which could lead to the 7-substituted pyrazolo[1,5-a]pyrimidine **2.14**.



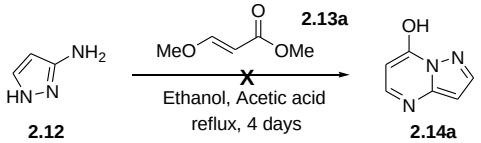
2.14 Y= OH, NH_2

2.13 R_1= Me, Et; R_2= CN, COOMe

2.12

Scheme 1. Retrosynthetic study of pyrazolo[1,5-a]pyrimidine.

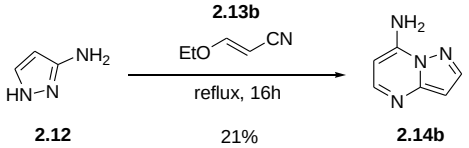
This method has been applied for the synthesis of the 7-hydroxy pyrazolo[1,5-a]pyrimidine **2.14a**, the 3-aminopyrazole has been condensed with methyl methoxy acrylate **2.13a** in refluxing ethanol with a catalytic amount of acetic acid, this reaction has been monitored from 3 hours to 4 days but no conversion was observed and the starting material **2.12** was remaining. In order to fully assess this reaction, another attempt has been done in refluxing acetic acid for 2 days but **2.12** remained not converted.



2.12 **2.14a**

Scheme 2. Condensation with methyl methoxy acrylate **2.13a**.

A different condensation reagent has been envisioned, **2.13a** was replaced by the ethoxy acrylonitrile **2.13b**. This reagent was used to afford the 7-amino derivative **2.14b**, the condensation reagent was used also as a solvent due to its low boiling-point (83°C) combined to a catalytic amount of acetic acid to perform the cyclization.



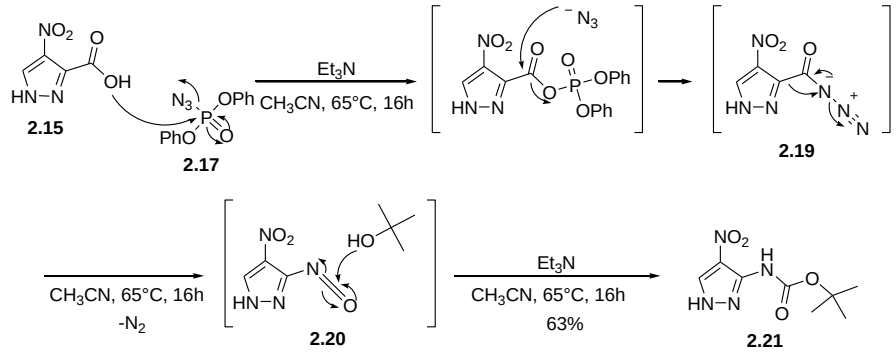
2.12 21% **2.14b**

Scheme 3. Condensation with ethoxy acrylonitrile.

2.14b was obtained as a white precipitate after 16 hours of reaction with a yield of 21%, most of the starting material **2.12** was not converted. The reaction time has been extended up to 3 days but did not give better yields, a possible explanation is that the conversion depends on the reaction temperature and another solvent with higher boiling point could lead to an increase of the yield.

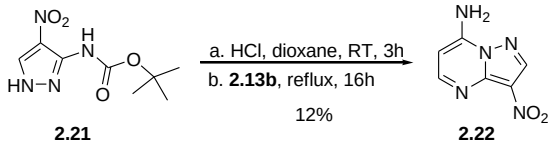
Before attempting to improve the yield of the condensation reaction leading to **2.14b**, it was decided to pursue the synthesis towards the desired product. The second part of the synthesis was the introduction of a second point of substitution at the position 3 of the pyrazolo[1,5-a]pyrimidine, in order to get a key intermediate with 2 functional groups which allow us to start a library synthesis. Following these principles, the introduction of a halide or a nitro group on the pyrazole ring may be of interest.

The condensation with ethoxy acrylonitrile **2.13b** could be applied to a functionalized aminopyrazole to lead directly to a versatile intermediate ready for a library synthesis. Our choice was directed to the commercially available pyrazole, 4-nitro-pyrazole-3-carboxylic acid **2.15**, this pyrazole contains a nitro group which can be the second functional group desired for the final scaffold. Prior to react with ethoxy acrylonitrile **2.13b**, the carboxylic acid has to be converted into the corresponding amine **2.16**, this transformation has been achieved by performing a Curtius rearrangement[5] using DPPA (diphenylphosphorylazide) **2.17** as azide source. The first step of this rearrangement was the formation of the intermediate **2.18**, then the azide group attacked **2.18** to generate an acyl azide **2.19**. Under thermal conditions, **2.19** was rearranged into an isocyanate **2.20** and then the Boc protected amine **2.21** was obtained by the reaction between **2.20** and *tert*-butanol leading to the carbamate **2.21**.



Scheme 4. Curtius rearrangement.

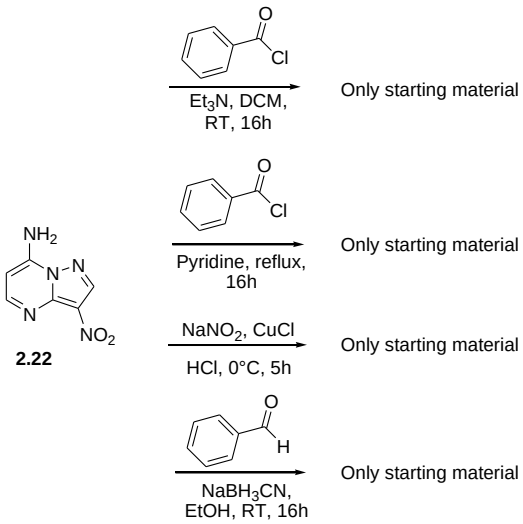
The protected aminopyrazole **2.21** became available and the rest of the synthesis to obtain the desired 3,7-disubstituted pyrazolo[1,5-a]pyrimidine **2.22** was the cleavage of the protective group under acidic conditions and the condensation with **2.13b**.



Scheme 5. Synthesis of the 7-amino-3-nitro-pyrazolo[1,5-a]pyrimidine **2.22**.

The condensation of the aminopyrazole after cleavage led to the 7-amino-3-nitro-pyrazolo[1,5-a]pyrimidine **2.22** as predicted but the yield was very low in our hands (12% after two steps).

The derivatisation of the key intermediate **2.22** started with an attempt of acylation with benzoyl chloride of the amine using triethylamine in CH_2Cl_2 at room temperature but after 16 hours of reaction, no conversion of the starting material has been observed, harsher conditions have been applied to the compound **2.22** to perform the acylation in refluxing pyridine, but the starting material remained intact. Instead of the acylation, a reductive amination has been attempted with benzaldehyde and sodium cyanoborohydride as reducer in ethanol at room temperature, after 16 hours we did not observed any conversion of the amine **2.22**. Finally, we decided to convert the amine into a halide to have access to another intermediate of interest, **2.22** has been treated with sodium nitrite in a mixture of water and hydrochloric acid at 0°C to obtain the corresponding diazonium salt and then by addition of CuCl to get the chloro derivative, but **2.22** was not reactive to the Sandmeyer reaction[6] and only the starting material was recovered after 5 hours of reaction.



Scheme 6. Different reactions on 7-amino-3-nitro-pyrazolo[1,5-a]pyrimidine.

One of the possible explanation is the presence of the electron withdrawing nitro group at the position 3 of **2.22** which makes the amine less reactive to further modification. It is possible also that the amine at the position 7 forms some hydrogen bonding which the nitrogen involved in the cyclization at the position 1, this hydrogen bond may prevent anymore derivatisation. Due to the unreactivity of the 7-amino-3-nitro-pyrazolo[1,5-a]pyrimidine **2.22**, another approach has been studied.

The introduction of a hydroxy group instead of an amine at the position 7 could be more interesting for further modifications on the scaffold and avoid the problem of reactivity associated to the amine, from this statement many efforts have been made to achieve the synthesis of the 7-hydroxy-pyrazolo[1,5-a]pyrimidine using a different method than a condensation with methyl methoxy acrylate **2.13a** discussed previously.

In 2006, Gavrin *et al.* from Wyeth Research[7] described several methodology for the synthesis of the two pyrazolo[1,5-a]pyrimidine regioisomers **2.14b** and **2.14c**.

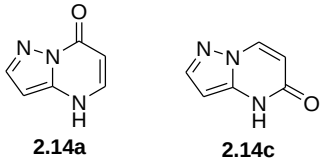
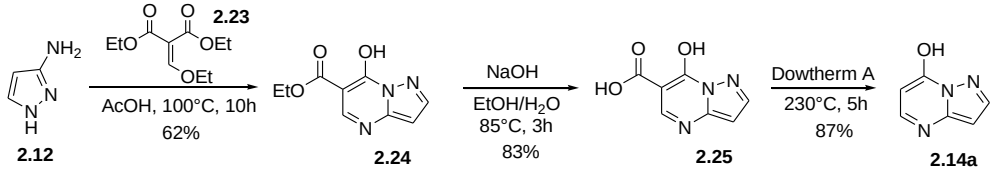


Figure 7. Pyrazolo[1,5-a]pyrimidine regioisomers.

The 7-hydroxy derivative **2.14a** has been synthesized in three steps starting from the 3-aminopyrazole **2.12**. The first step was the condensation of **2.12** with diethyl ethoxymethylene malonate **2.23** in refluxing acetic acid to obtain the desired pyrazolo[1,5-a]pyrimidine **2.24** containing the hydroxy group at the position 7 but also

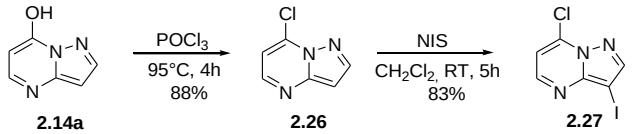
an ester group at the position 6, then it was necessary to do a saponification with sodium hydroxide to lead to the corresponding carboxylic acid **2.25**. A final decarboxylation with Dowtherm A at 230°C for 5 hours was performed to deliver the expected scaffold **2.14a**.



Scheme 7. Synthesis of the 7-hydroxy-pyrazolo[1,5-a]pyrimidine **2.14a**.

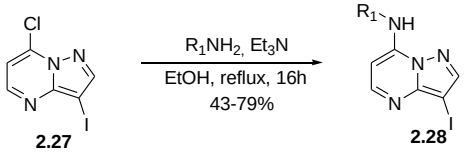
Compound **2.14a** has been subjected to chlorination in refluxing phosphorus oxychloride to assess the reactivity of the functional group, and the chlorinated derivative **2.26** was obtained in good yields. Hence, the introduction of second point of substitution has been applied directly to compound **2.26** instead of to repeat the synthesis with a functionalized pyrazole compared to previously discussed attempts.

The iodination of **2.26** was the first choice for a palladium cross-coupling strategy on the pyrazole moiety, the chlorinated derivative has been treated with N-iodo succinimide in dichloromethane at room temperature to afford the 7-chloro-3-iodo-pyrazolo[1,5-a]pyrimidine **2.27**, an intermediate ready to be used for the synthesis of a library.



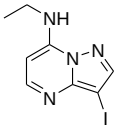
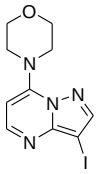
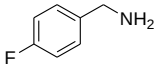
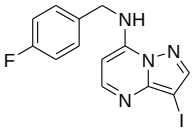
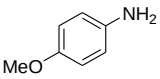
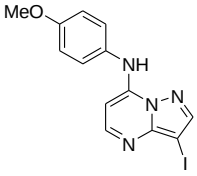
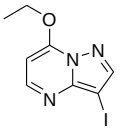
Scheme 8. Synthesis of the 7-chloro-3-iodo-pyrazolo[1,5-a]pyrimidine **2.27**.

The first strategy explored started with a nucleophilic substitution of **2.27**, different amines have been used such as aliphatic amine, cycloalkyl amine, aromatic amine and also an example of aliphatic alcohol. The substitution of the chloride at the position 7 of **2.27** led to 5 examples of amino (or alkoxy) substituted pyrazolo[1,5-a]pyrimidines **2.28** with good yield by refluxing the nucleophile, **2.27** and triethylamine in ethanol for 16 hours.



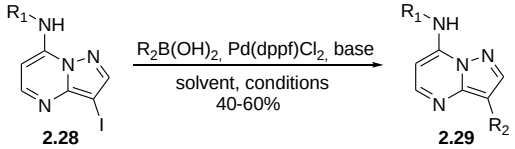
Scheme 9. Nucleophilic substitution.

Table 1. Substrate scope of the nucleophilic substitution.[a]

entry	starting material	nucleophile	pyrazolo[1,5-a]pyrimidine		yield (%)[b]
1	**2.27**			**2.28a**	79
2	**2.27**			**2.28b**	68
3	**2.27**			**2.28c**	61
4	**2.27**			**2.28d**	43
5	**2.27**			**2.28e**	57

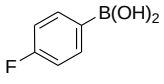
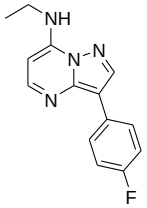
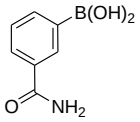
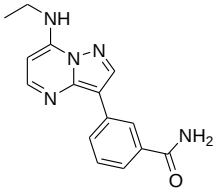
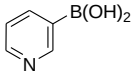
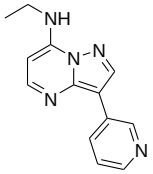
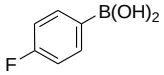
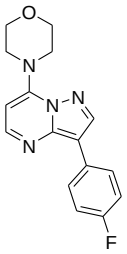
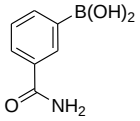
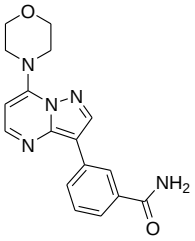
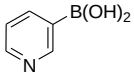
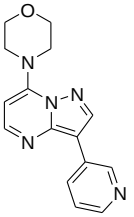
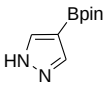
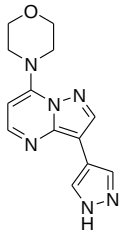
[a]All reactions were performed using 1 mmol of **2.27**, 1.1 mmol of amine and 181 μL of triethylamine in refluxing ethanol until completion of the reaction monitored by TLC. [b]Isolated yield.

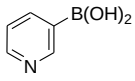
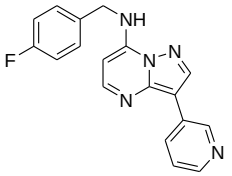
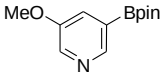
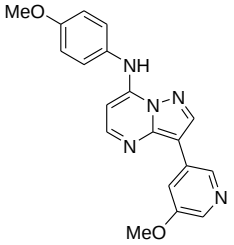
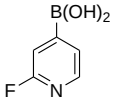
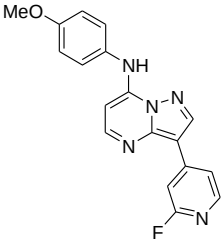
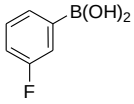
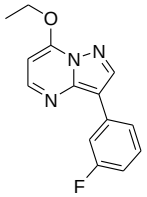
A set of 5 amino substituted derivatives **2.28** became available and the second part of the synthesis was based on a Suzuki[8] palladium cross-coupling strategy, in fact a numbers of boronic acids or esters have been chosen to react with **2.28** under two different conditions either thermal conditions or microwave conditions. Another difference between the two methods used was the nature of the base, for the coupling under conventional heating, K_3PO_4 has been chosen and while the reaction was performed under microwave irradiation, triethylamine was the base. The same catalyst 1,1'-bis(diphenylphosphino)ferrocene dichloro palladium has been selected for all the Suzuki coupling with **2.28** as starting material. A series of novel pyrazolo[1,5-a]pyrimidine derivatives **2.29** has been obtained with this strategy.



Scheme 10. Suzuki cross-coupling reaction.

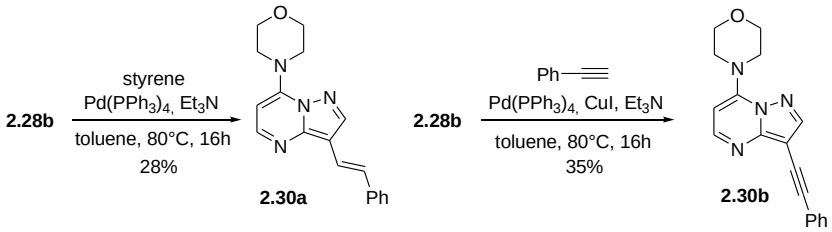
Table 2. Substrate scope of the Suzuki coupling.[a]

entry	starting material	organoboron reagent	pyrazolo[1,5-a]pyrimidine		yield (%)[b]
1	**2.28a**			**2.29a**	60[c]
2	**2.28a**			**2.29b**	43[d]
3	**2.28a**			**2.29c**	45[d]
4	**2.28b**			**2.29d**	58[c]
5	**2.28b**			**2.29**[e]	42[d]
6	**2.28b**			**2.29f**	51[d]
7	**2.28b**			**2.29g**	40[d]

8	**2.28c**			**2.29h**	43[d]
9	**2.28d**			**2.29i**	50[d]
10	**2.28d**			**2.29j**	40[d]
11	**2.28e**			**2.29k**	57[c]

[a]Two methods of Suzuki coupling were used. [b]Isolated yield. [c]Reactions were performed using 1 mmol of **2.28**, 1.1 mmol of organoboron reagent, 41 mg of Pd(dppf)Cl_2 and 467 mg of K_3PO_4 in refluxing dioxane using conventional heating conditions for 16 hours. [d]Reactions were performed using 1 mmol of **2.28**, 1.1 mmol of organoboron reagent, 41 mg of Pd(dppf)Cl_2 and 278 µL of triethylamine in 5 mL of water and dioxane (1/4), the vial was irradiated by microwaves at 105°C for 30minutes.

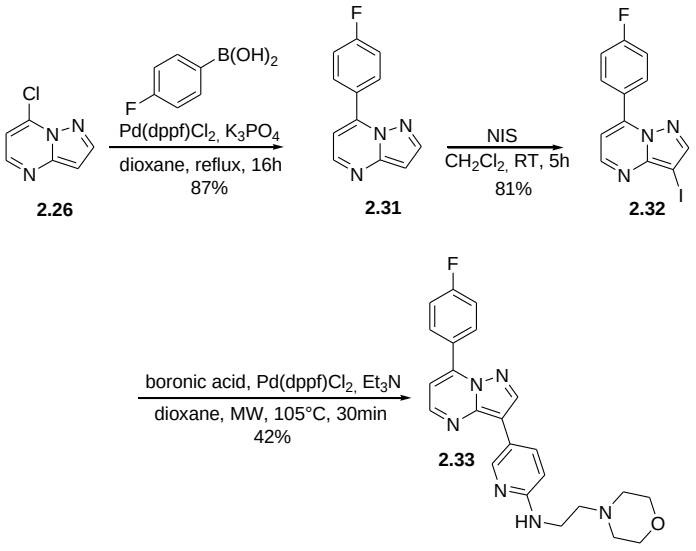
The development of a 11-members library of pyrazolo[1,5-a]pyrimidine derivatives **2.29** has been elaborated based on this first pathway, the first point of substitution on the pyrimidine side has been linked to various groups by nucleophilic substitution and then the introduction of a number of (hetero)aromatic rings has been attached by performing a Suzuki cross-coupling on the pyrazole moiety. In order to cover others palladium cross-coupling, the derivative **2.28b** has been coupled with styrene by a Heck reaction[9] to obtain an analogue containing an olefin bond **2.30a** and with phenyl acetylene leading to a derivative containing an alkyne bond **2.30b** by a Sonogashira coupling.[10] For these two coupling reactions, the catalysts used were identical, in fact tetrakis(triphenylphosphine) palladium was used alone for the Heck reaction and in combination with copper iodide for the Sonogashira reaction. The yields obtained were low but no further optimization has been done to improve the results.



Scheme 11. Heck and Sonogashira coupling reactions.

A second strategy has been explored, instead of to introduce a substituent by nucleophilic substitution at the position 7 of pyrazolo[1,5-a]pyrimidine **2.27**, a pathway based on a palladium cross-coupling strategy on the two halides could be of interest.

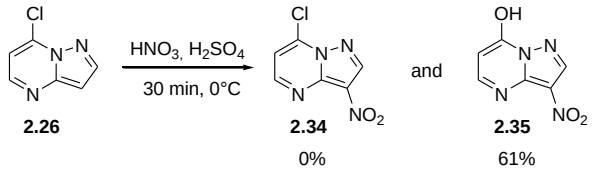
The iodine on the pyrazole ring of the scaffold should be the most reactive halide for a Suzuki reaction, but the high reactivity[11] of the chloride at the position 4 of the pyrimidine ring towards nucleophilic substitution or coupling directed us to search for another strategy to avoid possible problems of competition between the two halides during the reaction. In fact, the derivative **2.26** was more appropriate for the synthesis of library based on palladium cross coupling strategy, the 7-chloro-pyrazolo[1,5-a]pyrimidine **2.26** was subjected to a Suzuki coupling with *p*-fluorophenyl boronic acid under conventional heating to afford the derivative **2.31** in 87% yield after 16 hours. **2.31** was converted into the iodo derivative **2.32** to perform another Suzuki coupling under microwaves irradiation to obtain the product **2.33**.



Scheme 12. Synthesis of pyrazolo[1,5-a]pyrimidine **2.33**.

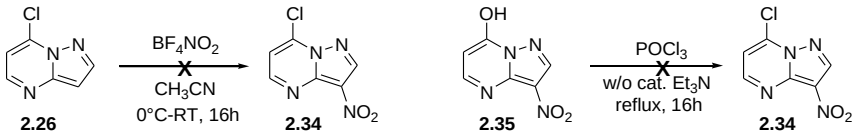
Most of the derivatives prepared so far were containing (hetero)aryl rings introduced by a Suzuki coupling on the pyrazole moiety of the pyrazolo[1,5-a]pyrimidine, some

efforts have been made to be able to introduce a nitrogen linker at the position 3 of the scaffold. The method envisioned to attach such linker was the nitration,[12] the application of this reaction to the compound **2.26** has been attempted but **2.34** was not observed, instead the derivative **2.35** was obtained as a green precipitate in 61% yield after work-up. A possible explanation could be that the introduction of a strong electron withdrawing group such as nitro group made the chloride at the position 7 of the scaffold more reactive towards nucleophilic substitution and has been substituted by water during the work-up leading to the **2.35** instead of the expected derivative **2.34**.



Scheme 13. Synthesis of 7-hydroxy-3-nitro-pyrazolo[1,5-a]pyrimidine.

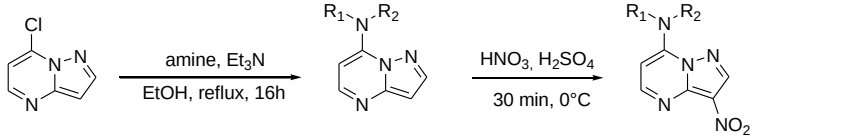
Following this possible explanation, compound **2.35** could prove the formation of **2.34** prior to work-up but while monitoring the reaction by LC/MS, only **2.35** has been observed. Milder conditions of nitration have been investigated using nitronium tetrafluoroborate[13] from 0°C to room temperature but the starting material **2.26** remained not converted after 16 hours of reaction. In parallel, **2.35** has been treated in refluxing phosphorus oxychloride during 16 hours to perform a chlorination of the hydroxy group, unfortunately **2.35** was not converted into **2.34**. The chlorination has been repeated with a catalytic amount of triethylamine[14] added to phosphorus oxychloride but the same results were obtained. These results led us to the same explanation previously discussed concerning the electron withdrawing nitro group attached to this scaffold.



Scheme 14. Attempts to synthesize 7-chloro-3-nitro-pyrazolo[1,5-a]pyrimidine **2.34**.

The aim of this methodology was to obtain two nitrogen linkers at the positions 3 and 7 of the pyrazolo[1,5-a]pyrimidine, but in view of our results, it was necessary to adapt the pathway. Compound **2.26** has been subjected to a nucleophilic substitution

prior to a nitration step with nitric acid in sulfuric acid to afford **2.37**. We were able to introduce aliphatic amine and cycloalkyl amine by SNar with this strategy.



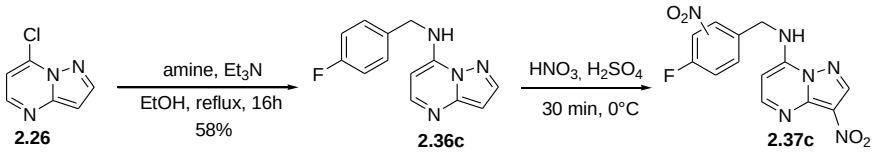
2.26

2.36a, R_1= Et, R_2= H, 68%
2.36b, R_1= R_2= Morpholine, 65%

2.37a, R_1= Et, R_2= H, 50%
2.37b, R_1= R_2= Morpholine, 57%

Scheme 15. Synthesis of 3-nitro-pyrazolo[1,5-a]pyrimidine derivatives **2.37**.

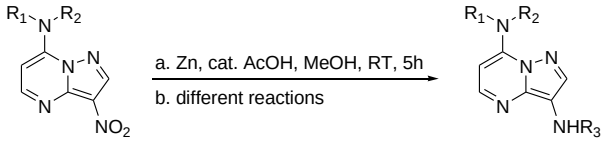
The introduction of (hetero)aryl or benzyl groups was not possible due to possible over nitration, in fact while attempting the nitration of **2.36c**, we observed by LC/MS the introduction of two nitro groups on the structure **2.37c**, one on the pyrazolo[1,5-a]pyrimidine at the position 3 and another one on the benzyl group of the substituent at the position 7, we believed that the second nitro was not attached to the scaffold itself due to the deactivation towards electrophilic aromatic substitution[15] after the introduction of the first nitro group.



2.26 **2.36c** **2.37c**

Scheme 16. Over nitration of the pyrazolo[1,5-a]pyrimidine derivatives **2.37c**.

The substituted 3-nitro-pyrazolo[1,5-a]pyrimidine analogue **2.37** was then reduced with zinc[16] and a catalytic amount of acetic acid in methanol to the corresponding amine which was used directly without further purification, the amine was subjected to different reactions in order to build a library of pyrazolo[1,5-a]pyrimidine derivatives **2.38**.

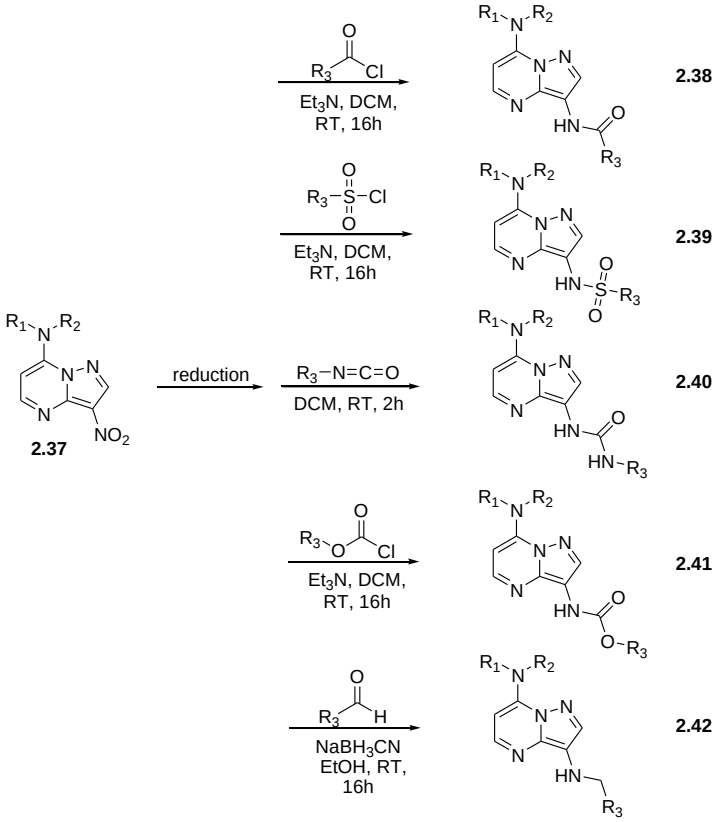


2.37 **2.38-2.42**

Scheme 17. Synthesis of pyrazolo[1,5-a]pyrimidine derivatives **2.38-2.42**.

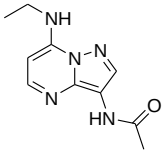
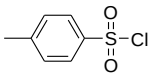
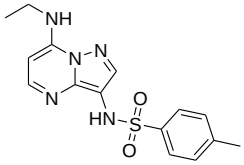
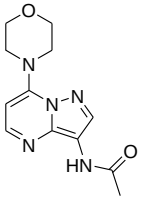
The amino derivative has been modified following five different reactions, the first one was the acylation[17] with acid chloride and triethylamine in dichloromethane for 16 hours leading to the amide derivatives **2.38**. Using the same conditions than the acylation with a sulfonyl chloride reagent led to a sulfonamide[18] derivative **2.39**. In order to synthesize some urea analogues[19] **2.40**, the amino derivative was treated with isocyanate species in dichloromethane for 2 hours. The synthesis of carbamates **2.41** has

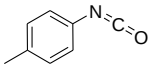
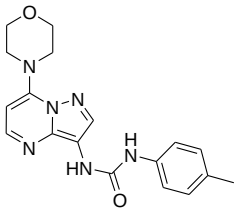
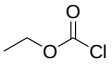
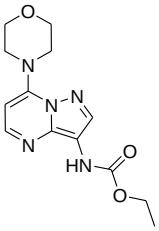
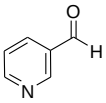
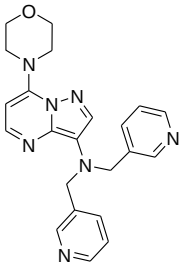
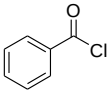
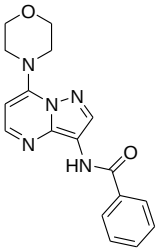
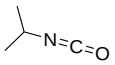
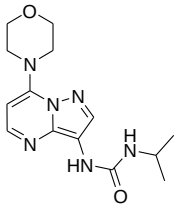
been achieved by reacting substituted chloroformates and the 3-amino-pyrazolo[1,5-a]pyrimidine. The last reaction used to develop this library was the reductive amination[20] to afford substituted amines **2.42**.



Scheme 18. Synthesis of pyrazolo[1,5-a]pyrimidine derivatives **2.38**-**2.42**.

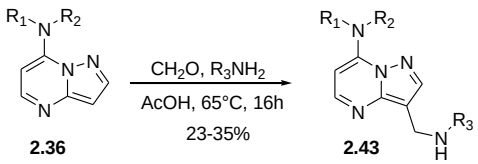
Table 3. Synthesis of pyrazolo[1,5-a]pyrimidine derivatives **2.38**-**2.42**.[a]

entry	starting material	reagent	pyrazolo[1,5-a]pyrimidine		yield (%)[b]
1	**2.37a**			**2.38a**	32
2	**2.37a**			**2.39a**	27
3	**2.37b**			**2.38b**	29

4	**2.37b**			**2.40a**	12
5	**2.37b**			**2.41a**	21
6	**2.37b**			**2.42a**	23
7	**2.37b**			**2.38c**	31
8	**2.37b**			**2.40b**	26

[a]See the experimental section for details. [b]Isolated yield.

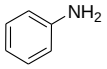
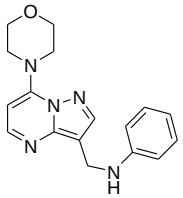
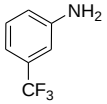
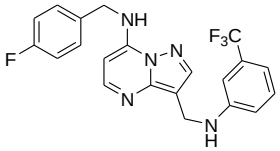
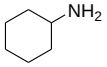
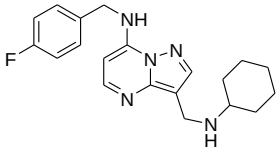
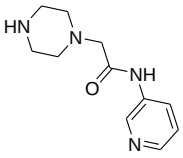
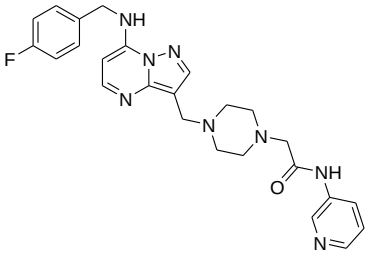
A last part was devoted to the synthesis of new analogues by Mannich reaction,[21] in fact the position 3 of the scaffold is very reactive towards electrophilic substitution. Hence, it was necessary to form an electrophilic reagent, with the Mannich reaction, the generation of such intermediates was possible by reacting a solution of formaldehyde and an amine to obtain an imine which was attacked by the pyrazolo[1,5-a]pyrimidine leading to **2.43**, this reaction was a one-pot procedure.



2.36 **2.43**

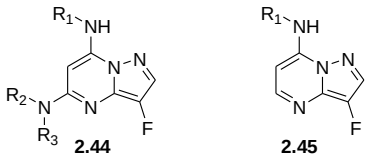
Scheme 19. Mannich reaction.

Table 4. Synthesis of pyrazolo[1,5-a]pyrimidine derivatives **2.43** by Mannich reaction.[a]

entry	starting material	amine	pyrazolo[1,5-a]pyrimidine		yield (%)[b]
1	**2.36b**			**2.43a**	35
2	**2.36c**			**2.43b**	29
3	**2.36c**			**2.43c**	33
4	**2.36c**			**2.43d**	23

[a]All reactions were performed using 1 mmol of **2.36**, 1.5 mmol of amine and 245 μL of 37% aqueous solution of formaldehyde in acetic acid at 65°C until completion of the reaction monitored by TLC. [b]Isolated yield.

Teijin pharma,[22] a Japanese pharmaceutical company described in 2004, some pyrazolo[1,5-a]pyrimidine derivatives as putative kinase inhibitors. Most of the analogues were containing fluorine at the position 3 of the pyrazole moiety. All the examples were substituted at the positions 5 and 7 by various amines affording compound such as **2.44**. Inspired by the work done by Teijin Pharma, we decided to direct our strategy into the synthesis of fluorinated pyrazolo[1,5-a]pyrimidine **2.45**.

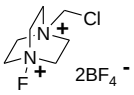


2.44 **2.45**

Figure 8. Fluorinated pyrazolo[1,5-a]pyrimidine derivatives.

One of the most common methods for the synthesis of fluorinated pyrazolo[1,5-a]pyrimidine is the fluorination of the scaffold directly by electrophilic fluorination,[23]

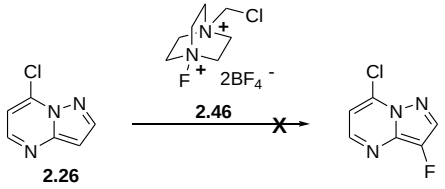
using a reagent like 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) also known as Selectfluor **2.46**.



2.46

Figure 9. Selectfluor, electrophilic fluorinating reagent.

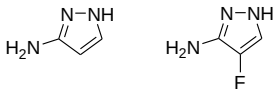
However, such fluorination method is generally low yielding and has never worked in the case of the 7-chloro pyrazolo[1,5-a]pyrimidine **2.26** in our hands, in contrast to other halogenations such as iodination discussed previously. An alternative procedure was envisioned to avoid this problem, the introduction of the fluorine at an earlier stage of synthesis. In fact, the synthesis of an intermediate containing a fluorine could be of value for the development of a simple approach to the synthesis of pyrazolo[1,5-a]pyrimidine derivatives.



2.26

Scheme 20. Fluorination of pyrazolo[1,5-a]pyrimidine.

The replacement of 3-amino-pyrazole **2.12** by 3-amino-4-fluoro-pyrazole **2.47** for the synthesis of the scaffold was first envisioned, some efforts have been made to synthesize the fluorinated pyrazole **2.47** but none of the methods attempted led to the desired target.



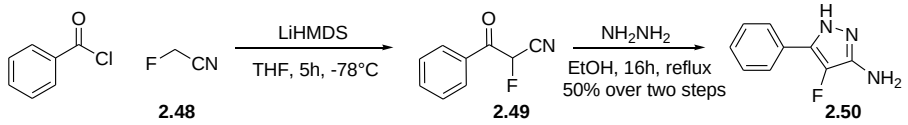
2.12 **2.47**

Figure 10. Pyrazole derivatives.

Finally, we decided to synthesize a fluorinated pyrazole substituted at the position 5 in contrast to **2.47**. It was possible to obtain the pyrazole **2.50** by using the method developed by Cocconcelli *et al.*,[24] this method allowed the synthesis of the fluoro derivative **2.50** through the intermediate α-fluoro-β-ketonitrile **2.49**.

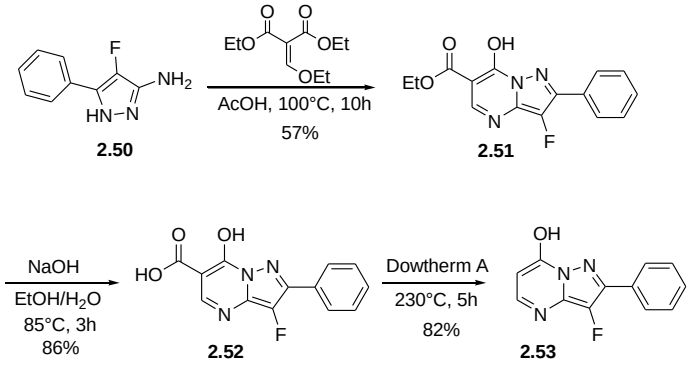
The substitution at the position 5 of the pyrazole ring was introduced with an acid chloride, in order to assess this strategy the benzoyl chloride was selected as model. The starting material of this pathway was the fluoro acetonitrile **2.48**, an acylation reaction

was first performed at -78°C with LiHMDS as the base and benzoyl chloride for 5 hours to afford the intermediate α-fluoro-β-ketonitrile **2.49** which was used without further purification in the next step. The hydrazinolysis of **2.49** in refluxing ethanol for 16 hours leading to the 3-amino-4-fluoro-5-phenyl-pyrazole **2.50** was straightforward with 50% yield over two steps.



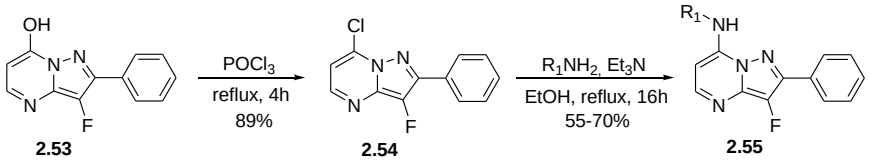
Scheme 21. Synthesis of 3-amino-4-fluoro-5-phenyl-pyrazole **2.50**.

The formation of the fluorinated pyrazolo[1,5-a]pyrimidine scaffold was then realized by condensation of **2.50** with diethyl ethoxymethylene malonate in acetic acid at 100°C for 10h to afford the pyrazolo[1,5-a]pyrimidine **2.51** in good yield, which after saponification with sodium hydroxide gave the carbolylic acid **2.52**. A final decarboxylation with Dowtherm A at 230°C for 5h, led to the desired intermediate **2.53**.



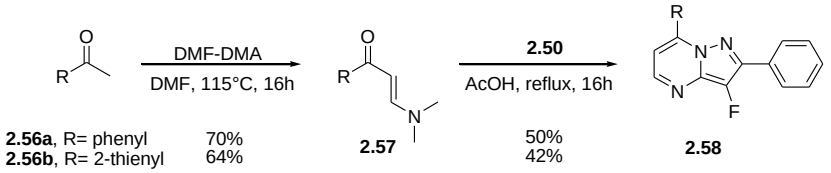
Scheme 22. Synthesis of the 3-fluoro-7-hydroxy-2-phenyl-pyrazolo[1,5-a]pyrimidine **2.53**.

Intermediate **2.53** was treated in refluxing phosphorus oxychloride to give the chlorinated derivative **2.54** which was subjected to nucleophilic substitution with different amines to obtain compound **2.55**. The versatility of this approach was demonstrated by the synthesis of a small-size library.



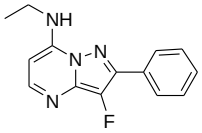
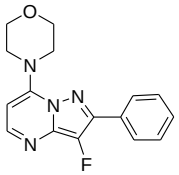
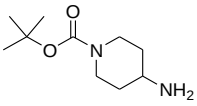
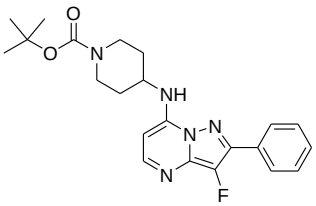
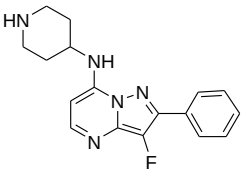
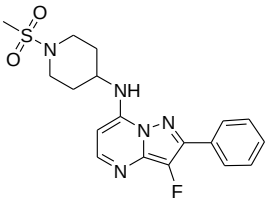
Scheme 23. Synthesis of 3-fluoro-pyrazolo[1,5-a]pyrimidine derivatives **2.55**.

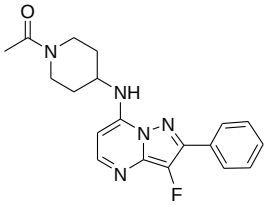
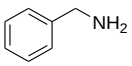
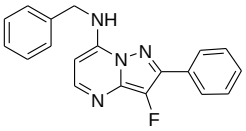
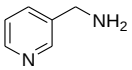
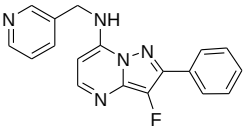
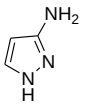
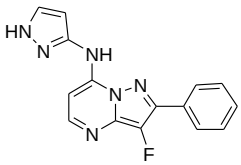
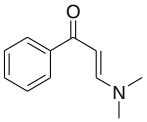
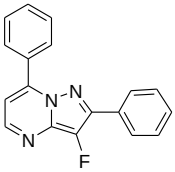
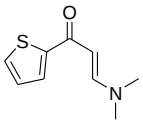
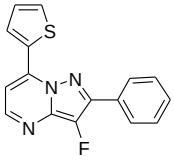
The amines used during the last step varied from aliphatic amines, cycloalkyl amines, benzyl amines to heteroaryl amines. In order to cover other substitutions at the position 7, another method has been used to obtain pyrazolo[1,5-a]pyrimidine derivatives directly attached to (hetero)aryl groups without a nitrogen linker. The 3-amino-4-fluoro-5-phenyl-pyrazole **2.50** has been condensed with (hetero)aryl dimethylamino propenone **2.57** (obtained from acetophenone derivatives **2.56**) in refluxing acetic acid to obtain 3-fluoro-7-(hetero)aryl-2-phenyl-pyrazolo[1,5-a]pyrimidine derivatives **2.58**.



Scheme 24. Synthesis of 3-fluoro-pyrazolo[1,5-a]pyrimidine derivatives **2.58**.

Table 5. Synthesis of 3-fluoro-2-phenyl-pyrazolo[1,5-a]pyrimidine derivatives.

entry	starting material	reagent	pyrazolo[1,5-a]pyrimidine		yield (%)[a]
1	**2.54**			**2.55a**	70[b]
2	**2.54**			**2.55b**	63[b]
3	**2.54**			**2.55c**	69[b]
4	**2.55c**	TFA/CH_2Cl_2 (1/4)		**2.55d**	65[c]
5	**2.55d**			**2.55e**	60[d]

6	**2.55d**			**2.55f**	58[d]
7	**2.54**			**2.55g**	68[b]
8	**2.54**			**2.55h**	58[b]
9	**2.54**			**2.55i**	55[b]
10	**2.50**			**2.58a**	50[e]
11	**2.50**			**2.58b**	42[e]

[a]Isolated yield. [b]All reactions were performed using 1 mmol of **2.54**, 1.1 mmol of amine and 181 µL of triethylamine in refluxing ethanol until completion of the reaction monitored by TLC. [c]Cleavage of Boc group has been done by stirring 1 mmol of **2.55c** in 5 mL of TFA/CH_2Cl_2 (1/4) from 0°C to room temperature for 2 hours. [d]All reactions were performed using 1 mmol of **2.55c**, 1.05 mmol of acid chloride or sulfonyl chloride and 181 µL of triethylamine in CH_2Cl_2 at room temperature for 16 hours. [e]All reactions were performed using 1 mmol of **2.50** and 1.1 mmol of condensation reagent in refluxing acetic acid for 16 hours.

3. Biological evaluation

The library of pyrazolo[1,5-a]pyrimidine derivatives described during this chapter has been screened towards selected kinases of ongoing projects of Galapagos. The compounds were first tested in an enzymatic assay at constant concentration and for the analogues displaying an interesting percentage of inhibition for a specified kinase, a further assay was performed at different concentrations in order to obtain the half maximal inhibitory concentration IC_{50}.

Table 6. Biological evaluation of pyrazolo[1,5-a]pyrimidine derivatives (N/A not active).

entry	compounds	LRRK2 (% inhibition at 10 μM)	IC_{50} (nM)	MAP4K4 (% inhibition at 10 μM)	IC_{50} (nM)
1	**2.29a**	81	>4000	78	>4000
2	**2.29b**	91	2180	98	859
3	**2.29c**	65	>4000	54	>4000
4	**2.29d**	N/A	N/A	N/A	N/A
5	**2.29e**	N/A	N/A	N/A	N/A
6	**2.29f**	N/A	N/A	N/A	N/A
7	**2.29g**	N/A	N/A	N/A	N/A
8	**2.29h**	N/A	N/A	N/A	N/A
9	**2.29i**	N/A	N/A	N/A	N/A
10	**2.29j**	N/A	N/A	65	>4000
11	**2.29k**	N/A	N/A	N/A	N/A
12	**2.30a**	78	>4000	84	>4000
13	**2.30b**	N/A	N/A	N/A	N/A
14	**2.33**	N/A	N/A	N/A	N/A
15	**2.38a**	53	>4000	N/A	N/A
16	**2.38b**	N/A	N/A	N/A	N/A
17	**2.38c**	N/A	N/A	N/A	N/A
18	**2.39a**	N/A	N/A	N/A	N/A
19	**2.40a**	N/A	N/A	N/A	N/A
20	**2.40b**	N/A	N/A	N/A	N/A
21	**2.41a**	N/A	N/A	56	>4000
22	**2.42a**	N/A	N/A	N/A	N/A
23	**2.43a**	N/A	N/A	N/A	N/A
24	**2.43b**	N/A	N/A	N/A	N/A
25	**2.43c**	N/A	N/A	N/A	N/A
26	**2.43d**	N/A	N/A	N/A	N/A

entry	compounds	CDK9 (% inhibition at 10 μM)	IC_{50} (nM)	TGF-β II (% inhibition at 10 μM)	IC_{50} (nM)
27	**2.55a**	8	N/A	14	N/A
28	**2.55b**	7	N/A	13	N/A
29	**2.55c**	N/A	N/A	N/A	N/A
30	**2.55d**	6	N/A	7	N/A
31	**2.55e**	N/A	N/A	N/A	N/A
32	**2.55f**	N/A	N/A	N/A	N/A
33	**2.55g**	18	N/A	32	N/A
34	**2.55h**	6	N/A	26	N/A
35	**2.55i**	7	N/A	71	N/A
36	**2.58a**	N/A	N/A	N/A	N/A
37	**2.58b**	N/A	N/A	18	N/A

The screening of the derivatives based on the pyrazolo[1,5-a]pyrimidine scaffold led to the discovery of one analogue **2.29b** displaying a half maximal inhibitory concentration IC_{50} of 859 nM towards MAP4K4. The compounds containing a cycloalkylamine such as morpholine did not show significant inhibition, in fact it seems better to introduce a small aliphatic group such as ethyl at the position 7 of the scaffold to be able to inhibit the kinase (Table 6, entry 1-3). While varying the linker, the O-linked scaffold **2.29k** was not active compared to the N-linked analogues. The introduction of a fluoride on the pyrazolo[1,5-a]pyrimidine was expected to improve the biological activity of the synthesized analogues. The compounds were screened towards CDK9 and TGF-β II to evaluate the percentage of inhibition but only the compound **2.55i** showed 71 % of inhibition at a concentration of 10 μM against TGF-β II receptor.

4. Conclusion

In summary, we have first synthesized the 7-hydroxy-pyrazolo[1,5-a]pyrimidine following the method developed by Gavrin *et al*., hence the elaboration of a key intermediate allowing the synthesis of a library has been done by introducing two halides at the positions 3 and 7 of the scaffold. Then a first strategy based on a sequence of nucleophilic substitution/Suzuki coupling gave a 11-member library, two examples have been added to cover others palladium cross-coupling reactions such as Heck and Sonogashira reactions.

A second part was devoted to the development of a pyrazolo[1,5-a]pyrimidine library with a nitrogen linker at the positions 3 and 7, different attempts of nitration have been done on the chloro derivative but we never obtained the expected derivative. A nucleophilic substitution has been done before the nitration of the scaffold, two examples of 3-nitro-pyrazolo[1,5-a]pyrimidine analogues have been synthesized. This method had limitations because it was not possible to perform a mono nitration while the scaffold was substituted by aryl or benzyl amines, in fact overnitration was observed. The derivatives with the nitro group were reduced to the corresponding amines with zinc prior to build a library by using various reactions such as acylation or reductive amination.

The pyrazolo[1,5-a]pyrimidine is known to be a good substrate for electrophilic aromatic substitution, 4 analogues have been synthesized by performing a Mannich reaction with formaldehyde and different amines.

In the last part, we have elaborated a simple approach for the synthesis of fluorinated pyrazolo[1,5-a]pyrimidine derivatives by introducing the fluorine at an earlier stage of the synthesis instead of using a direct electrophilic fluorination on the scaffold such as Selectfluor. The method could be employed to the development of a small-size library of “drug-like” compounds with different amines.

5. Experimental section

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 ºC) under a nitrogen or argon atmosphere. Reaction temperatures are reported as bath temperature. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Compounds were visualized with UV light (λ = 254 nm). Products were purified by flash chromatography on ICN silica gel 63-200, 60 Å. Melting points were obtained on a melting point apparatus Electrothermal IA9200 with open capillary tubes. ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz, 500 MHz and 600 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as the solvent. The ^{1}H and ^{13}C chemical shifts were referenced to residual solvent signals at δ H/C 7.26/77.00 ($CDCl_3$), 3.31/49.10 and 2.50/39.50 (DMSO-d_6) relative to TMS as internal standard. Coupling constants *J* [Hz] were directly taken from the spectra. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High resolution mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Electrospray MS spectra were obtained on a Micromass platform LC/MS spectrometer. Column used for all LC/MS analysis: Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm ID x 50 mm L. All the methods are using MeCN/H_2O gradients. Water contains either 0.1 % TFA or 0.1 % NH_3.

Pyrazolo[1,5-a]pyrimidin-7-amine (2.14b)

A solution of compound **2.12** (3 g, 36.11 mmol) in 15 mL of ethoxy acrylonitrile **2.13b** was refluxed for 16 hours, after which was allowed to cool to room temperature and the formation of a precipitate was observed. The precipitate was filtered and washed with Et_2O to afford the desired compound **2.14** in 21% yield as beige solid. Data for **2.14b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83-7.87 (m, 2H), 6.37 (d, *J* = 5.9 Hz, 1H), 5.65 (d, *J* = 5.6 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.7, 141.4, 138.1, 136.7, 93.4, 87.1.

***Tert*-butyl 4-nitro-1H-pyrazol-3-ylcarbamate (2.21)**

A solution of compound **2.15** (2 g, 12.73 mmol) in 15 mL of acetonitrile under argon was successively added triethylamine (4.4 mL, 31.83 mmol), DPPA **2.17** (3 mL, 14.00 mmol) and *tert*-butanol (1.3 mL, 14.00 mmol) at room temperature. The reaction mixture was refluxed for 16 hours and after completion of the reaction, was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the protected pyrazole **2.21** in 63% yield as white solid. Data for **2.21**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.65 (br. s, 1H), 7.44 (s, 1H), 4.41 (br. s, 1H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.2, 147.4, 133.7, 125.2, 82.5, 26.9.

3-Nitropyrazolo[1,5-a]pyrimidin-7-amine (2.22)

To a solution of **2.21** (5 g, 43.06 mmol) in 15 mL of dioxane under argon at 0 °C was added 500 μL of concentrated hydrochloric acid and the reaction was stirred at room temperature for 3 hours. Addition of 10 mL of water and 15 mL of CH_2Cl_2 to the reaction mixture and the pH was adjusted to 8 with K_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to obtain the amino pyrazole intermediate as an oily solid which was used directly without further purification for the next step.

A solution of the previously prepared pyrazole in 15 mL of ethoxy acrylonitrile **2.13b** was refluxed for 16 hours, after which was allowed to cool to room temperature and the formation of a precipitate was observed. The precipitate was filtered and washed with Et_2O to afford the desired compound **2.22** in 12% yield as beige solid. Data for **2.22**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.12 (d, *J* = 7.8 Hz, 1H), 5.96 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.8, 149.3, 147.2, 139.9, 101.4.

Ethyl 7-Oxo-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (2.24)

To a solution of pyrazol-3-ylamine **2.12** (4.15 g, 50 mmol) in 25 mL of acetic acid under argon at room temperature was added diethyl ethoxymethylenemalonate **2.23** (11.89 g, 55 mmol). The mixture was heated at 100 °C for 10 hours. The suspension was filtered warm, then washed several times with EtOH, and dried to yield **2.24** in 62% yield as white solid. Data for **2.24**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.10 (s, 1 H), 8.59 (s, 1 H), 7.93 (d, *J*=2.0 Hz, 1 H), 6.31 (d, *J*=2.0 Hz, 1 H), 4.23 (q, *J*=7.1 Hz, 2 H), 1.28 (t, *J*=7.1 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.6, 157.2, 145.3, 143.4, 140.9, 98.5, 91.2, 60.0, 14.3.

7-Oxo-4,7-dihydropyrazolo[1,5-α]pyrimidine-6-carboxylic acid (2.25)

To a suspension of **2.24** (2.07 g, 10 mmol) in 30 mL of ethanol and water (1/1) under argon at room temperature was added 2.5 N NaOH (10 mL). The resulting suspension was heated to 85 °C for 3 hours. The suspension was cooled to room temperature and diluted with water (100 mL) and concentrated hydrochlorhydric acid until the formation of a suspension (pH=1). The white suspension was stirred an additional 30 min, filtered, washed with water, and dried to yield **2.25** in 83% yield as white solid. Data for **2.25**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.95 (s, 1 H), 8.72 (s, 1 H), 8.05 (d, *J*=2.0 Hz, 1 H), 6.41 (d, *J*=2.0 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.7, 157.2, 145.7, 144.5, 141.0, 97.7, 91.9.

Pyrazolo[1,5-a]pyrimidin-7-ol (2.14a)

To the carboxylic acid **2.25** (1 g, 5.58 mmol) was added 10 mL of Dowtherm A. The suspension was heated to 230 °C for 5h. The reaction mixture was cooled to room temperature, then diluted with 30 mL of hexanes, stirred vigorously, filtered and dried to obtain the desired product **2.14a** in 87% yield as a light brown solid. Data for **2.14a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.36 (s, 1 H), 7.86-7.89 (m, 2 H), 6.18 (dd, *J*=1.8, 0.6 Hz, 1 H), 5.68 (d, *J*=7.3 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.5, 140.5, 139.8, 137.5, 93.1, 86.9.

7-Chloropyrazolo[1,5-a]pyrimidine (2.26)

2.14a (1 g, 7.40 mmol) was dissolved in 10 mL of phosphorus oxychloride at room temperature. The reaction mixture was heated at 95 °C for 4 hours after which the color of the solution became brown. The flask was allowed to cool to room temperature and the excess of phosphorus oxychloride was concentrated in vacuo to a brown oil. The oil was dissolved in 15 mL of EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated to afford crude **2.26** as brown oil used directly without further purification.

7-Chloro-3-iodopyrazolo[1,5-a]pyrimidine (2.27)

To a solution of chlorinated derivative **2.26** (1 g, 6.51 mmol) in 15 mL of CH_2Cl_2 under argon at room temperature was added NIS (1.61 g, 7.16 mmol). The mixture was stirred at ambient temperature for 5 hours. After completion of the reaction monitored by TLC, 10 mL of saturated aqueous $NaHCO_3$ was added. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the pyrazolo[1,5-a]pyrimidine **2.27** in 83% yield as beige solid. Data for **2.27**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (d, *J* = 5.6 Hz, 1H), 8.32 (s, 1H), 6.28 (d, *J* = 5.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.1, 150.7, 149.0, 146.5, 143.4, 95.9.

General procedure for the preparation of pyrazolo[1,5-a]pyrimidine derivatives 2.28:

To a solution of **2.27** (280 mg, 1 mmol) in 5 mL of EtOH under argon was added an amine (1.1 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 7:3) to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.28**.

N-ethyl-3-iodopyrazolo[1,5-a]pyrimidin-7-amine (2.28a)

Beige solid, 79% yield; Data for **2.28a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15-8.17 (m, 2H), 8.05 (t, *J* = 5.9 Hz, NH), 6.21 (d, *J* = 5.4 Hz, 1H), 3.40 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 151.4, 141.7, 146.2, 143.7, 86.5, 81.4, 37.1, 14.5.

3-Iodo-7-morpholinopyrazolo[1,5-a]pyrimidine (2.28b)

Orange solid, 68% yield; Data for **2.28b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (d, *J* = 5.1 Hz, 1H), 8.23 (s, 1H), 6.46 (d, *J* = 5.2 Hz, 1H), 3.79-3.81 (m, 4H), 3.72-3.74 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.4, 151.0, 150.2, 149.7, 147.2, 93.5, 65.7, 48.1.

N-(4-fluorobenzyl)-3-iodopyrazolo[1,5-a]pyrimidin-7-amine (2.28c)

Yellow solid, 61% yield; Data for **2.28c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (t, *J* = 6.4 Hz, 1H), 8.37 (s, 1H), 7.94 (d, *J* = 5.0 Hz, 1H), 7.39-7.43 (m, 2H), 7.15-7.18 (m, 2H), 6.11 (d, *J* = 5.1 Hz, 1H), 4.63 (d, *J* = 6.7 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.7, 161.0, 149.7, 148.9, 146.1, 139.9, 133.7, 122.0, 116.8, 93.3, 84.6, 46.5.

7-Ethoxy-3-iodopyrazolo[1,5-a]pyrimidine (2.28e)

Beige solid, 57% yield; Data for **2.28e**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.48 (d, *J* = 5.1 Hz, 1H), 8.23 (s, 1H), 6.67 (d, *J* = 5.1 Hz, 1H), 4.51 (q, *J* = 7.0 Hz, 2H), 1.48 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.1, 152.4, 149.5, 148.4, 88.6, 67.0, 49.7, 14.0.

General procedure for the preparation of pyrazolo[1,5-a]pyrimidine derivatives 2.29:

Method using conventional heating

To a solution of **2.28** (1.0 mmol) in 5 mL of dioxane under argon was added a boronic acid (1.3 mmol), K_3PO_4 (467 mg, 2.2 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction for 16 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the aryl substituted pyrazolo[1,5-a]pyrimidine **2.29**.

Method using microwaves irradiation

To a solution of **2.28** (1.0 mmol) in 5 mL of water and dioxane (1/4) under argon was added a boronic acid (1.3 mmol), triethylamine (348 µL, 2.5 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was irradiated by microwaves at 105°C for 30minutes and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the aryl substituted pyrazolo[1,5-a]pyrimidine **2.29**.

N-ethyl-3-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidin-7-amine (2.29a)

Brown solid, 60% yield; Data for **2.29a**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.22 (d, *J* = 5.3 Hz, 1H), 8.11-8.13 (m, 2H), 8.00 (t, *J* = 5.9 Hz, NH), 7.20 (t, *J* = 8.9 Hz, 2H), 6.22 (d, *J* = 5.3 Hz, 1H), 3.40-3.45 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.4, 162.8, 153.6, 150.3, 148.6, 144.5, 132.6, 130.4, 118.7, 109.9, 89.2, 39.7, 17.4.

3-(7-(Ethylamino)pyrazolo[1,5-a]pyrimidin-3-yl)benzamide (2.29b)

Beige solid, 43% yield; Data for **2.29b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.55-8.56 (m, 1H), 8.39 (d, *J* = 7.9 Hz, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 8.17 (t, *J* = 6.1 Hz, NH), 8.00 (s, 1H), 7.88-7.90 (m, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.39 (s, 1H), 6.27 (d, *J* = 5.4 Hz, 1H), 3.43-3.47 (m, 2H), 1.24 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 153.7, 150.4, 149.0, 144.7, 138.1, 136.4, 131.8, 131.3, 129.3, 127.6, 110.3, 89.3, 39.7, 17.5.

N-ethyl-3-(pyridin-3-yl)pyrazolo[1,5-a]pyrimidin-7-amine (2.29c)

Beige solid, 45% yield; Data for **2.29c**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.33-9.34 (m, 1H), 8.74 (s, 1H), 8.52-8.53 (m, 1H), 8.36-8.37 (m, 1H), 8.28 (d, *J* = 5.5 Hz, 1H), 8.22 (t, *J* = 5.9 Hz, NH), 7.40-7.42 (m, 1H), 6.29 (d, *J* = 5.4 Hz, 1H), 3.44-3.47 (m, 2H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 150.6, 147.1, 146.4, 146.1, 146.0, 141.3, 131.9, 129.1, 123.7, 104.2, 86.2, 36.4, 14.2.

3-(4-Fluorophenyl)-7-morpholinopyrazolo[1,5-a]pyrimidine (2.29d)

Brown solid, 58% yield; Data for **2.29d**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.6 (s, 1H), 8.39 (d, *J* = 5.0 Hz, 1H), 8.17-8.20 (m, 2H), 7.25 (t, *J* = 8.9 Hz, 2H), 6.47 (d, *J* = 5.1 Hz, 1H), 3.82-3.84 (m, 3H), 3.76-3.78 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.5, 159.6, 150.3, 150.2, 146.8, 141.3, 129.0, 127.4, 115.4, 106.9, 93.4, 65.7, 48.2.

3-(7-Morpholinopyrazolo[1,5-a]pyrimidin-3-yl)benzamide (2.29e)

Brown solid, 42% yield; Data for **2.29e**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.43 (s, 1H), 8.41 (d, *J* = 5.0 Hz, 1H), 8.24 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 6.19 (d, *J* = 5.0 Hz, 1H), 3.99-4.00 (m, 4H), 3.78-3.80 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.8, 152.9, 144.5, 132.6, 132.0, 128.0, 127.8, 96.1, 69.3, 51.5.

7-Morpholino-3-(pyridin-3-yl)pyrazolo[1,5-a]pyrimidine (2.29f)

White solid, 51% yield; Data for **2.29f**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.45-8.49 (m, 2H), 8.40-8.41 (m, 2H), 7.36-7.39 (m, 1H), 6.20 (d, *J* = 5.0 Hz, 1H), 3.99-4.01 (m, 4H), 3.78-3.80 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.8, 150.1, 147.2, 146.8, 141.1, 133.5, 128.7, 123.6, 106.3, 93.2, 66.3, 48.5.

7-Morpholino-3-(1H-pyrazol-4-yl)pyrazolo[1,5-a]pyrimidine (2.29g)

Brown solid, 40% yield; Data for **2.29g**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.43 (s, 1H), 8.33 (d, *J* = 5.1 Hz, 1H), 8.07-8.11 (m, 2H), 6.39 (d, *J* = 5.1 Hz, 1H), 3.81-3.83 (m, 4H), 3.73-3.75 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 150.1, 149.5, 146.2, 140.6, 111.9, 102.2, 92.8, 65.8, 48.1.

N-(4-fluorobenzyl)-3-(pyridin-3-yl)pyrazolo[1,5-a]pyrimidin-7-amine (2.29h)

Beige solid, 43% yield; Data for **2.29h**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.32-9.33 (m, 1H), 8.89 (t, *J* = 6.5 Hz, NH), 8.78 (s, 1H), 8.50-8.52 (m, 1H), 8.37-8.38 (m, 1H), 8.23 (d, *J* = 5.3 Hz, 1H), 7.46-7.49 (m,

2H), 7.40-7.42 (m, 1H), 7.17 (t, *J* = 8.9 Hz, 2H), 6.24 (d, *J* = 5.3 Hz, 1H), 4.64 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 164.0, 153.9, 150.5, 149.8, 149.6, 149.2, 144.8, 137.6, 135.4, 132.6, 132.3, 127.1, 118.7, 107.8, 90.2, 47.3.

3-(2-Fluoropyridin-4-yl)-N-(4-methoxyphenyl)pyrazolo[1,5-a]pyrimidin-7-amine (2.29j)

Brown solid, 40% yield; Data for **2.29j**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.02 (s, 1H), 8.38 (d, *J* = 5.4 Hz, 1H), 8.21 (d, *J* = 5.3 Hz, 1H), 8.15-8.16 (m, 1H), 7.95 (s, 1H), 7.39 (t, *J* = 8.1 Hz, 1H), 7.07-7.08 (m, 2H), 6.88-6.90 (m, 1H), 6.53 (d, *J* = 5.4 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.1, 163.5, 160.2, 151.9, 147.8, 147.1, 146.2, 146.1, 143.1, 138.2, 130.5, 117.7, 116.5, 111.9, 110.4, 104.5, 103.7, 89.3, 55.5.

7-Ethoxy-3-(3-fluorophenyl)pyrazolo[1,5-a]pyrimidine (2.29k)

Beige solid, 57% yield; Data for **2.29k**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.58 (d, *J* = 5.0 Hz, 1H), 8.05-8.08 (m, 1H), 8.02-8.03 (m, 1H), 7.46 (q, *J* = 7.9 Hz, 1H), 7.03-7.06 (m, 1H), 6.73 (d, *J* = 5.1 Hz, 1H), 4.55 (q, *J* = 7.0 Hz, 2H), 1.51 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.5, 161.9, 155.2, 152.2, 146.7, 142.9, 134.7, 130.6, 121.4, 112.3, 111.8, 107.3, 89.0, 66.9, 14.2.

7-Morpholino-3-styrylpyrazolo[1,5-a]pyrimidine (2.30a)

To a solution of **2.28b** (330 mg, 1.0 mmol) in 5 mL of toluene under argon was added styrene (229 mg, 2.0 mmol), tetrakis(triphenylphosphine) palladium (116 mg, 0.1 mmol) and triethylamine (236 μL, 1.7 mmol) at room temperature. The reaction mixture was heated at 80 °C for 16 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the pyrazolo[1,5-a]pyrimidine **2.30a** in 28% yield as beige solid. Data for **2.30a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.38 (d, *J* = 5.2 Hz, 1H), 8.18 (s, 1H), 7.49-7.53 (m, 2H), 7.31-7.36 (m, 3H), 6.78-6.86 (m, 2H), 6.35 (d, *J* = 5.0 Hz, 1H), 3.93-3.96 (m, 4H), 3.68-3.73 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.4, 150.7, 149.6, 145.3, 139.9, 132.1, 128.3, 128.0, 123.6, 117.0, 96.4, 66.7, 47.3.

7-Morpholino-3-(2-phenylethynyl)pyrazolo[1,5-a]pyrimidine (2.30b)

To a solution of **2.28b** (330 mg, 1.0 mmol) in 5 mL of toluene under argon was added phenyl acetylene (165 mg, 1.5 mmol), tetrakis(triphenylphosphine) palladium (116 mg, 0.1 mmol), copper iodide (19 mg, 0.1 mmol) and triethylamine (236 μL, 1.7 mmol) at room temperature. The reaction mixture was heated at 80 °C for 16 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the pyrazolo[1,5-a]pyrimidine **2.30b** in 35% yield as brown solid. Data for **2.30b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, *J* = 5.0 Hz, 1H), 8.22 (s, 1H), 7.57-7.59 (m, 2H), 7.30-7.35

(m, 3H), 6.17 (d, *J* = 5.1 Hz, 1H), 3.96-3.98 (m, 4H), 3.77-3.79 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.0, 150.9, 150.7, 145.8, 131.5, 128.2, 127.8, 123.7, 93.4, 93.1, 93.0, 79.6, 66.2, 48.5.

7-(4-Fluorophenyl)pyrazolo[1,5-a]pyrimidine (2.31)

To a solution of **2.26** (154 mg, 1.0 mmol) in 5 mL of dioxane under argon was added *p*-fluorophenyl boronic acid (182 mg, 1.3 mmol), K_3PO_4 (467 mg, 2.2 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction for 16 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the pyrazolo[1,5-a]pyrimidine **2.31** in 87% yield as beige solid. Data for **2.31**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (d, *J* = 4.4 Hz, 1H), 8.21-8.29 (m, 3H), 7.44-7.50 (m, 2H), 7.26 (d, *J* = 4.4 Hz, 1H), 6.84 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.2, 149.7, 149.5, 144.6, 132.3, 132.1, 115.8, 115.6, 107.7, 96.7.

7-(4-Fluorophenyl)-3-iodopyrazolo[1,5-a]pyrimidine (2.32)

To a solution of **2.31** (213 mg, 1.0 mmol) in 15 mL of CH_2Cl_2 under argon at room temperature was added NIS (270 mg, 1.2 mmol). The mixture was stirred at ambient temperature for 5 hours. After completion of the reaction monitored by TLC, 10 mL of saturated aqueous $NaHCO_3$ was added. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the pyrazolo[1,5-a]pyrimidine **2.32** in 81% yield as beige solid. Data for **2.32**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, *J* = 4.3 Hz, 1H), 7.92 (s, 1H), 7.82-7.86 (m, 2H), 6.99-7.05 (m, 2H), 6.77 (d, *J* = 4.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 150.8, 147.2, 141.9, 133.4, 130.9, 110.9, 107.3, 102.1, 94.9.

5-(7-(4-Fluorophenyl)pyrazolo[1,5-a]pyrimidin-3-yl)-N-(2-morpholinoethyl)pyridin-2-amine (2.33)

To a solution of **2.32** (339 mg, 1.0 mmol) in 5 mL of water and dioxane (1/4) under argon was added boronic acid (1.3 mmol), triethylamine (347 μL, 2.5 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was irradiated by microwaves at 105°C for 30minutes and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the aryl substituted pyrazolo[1,5-a]pyrimidine **2.33** in 42% yield as brown solid. Data for **2.33**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (d, *J* = 4.1 Hz, 1H), 7.87 (s, 1H), 7.79-7.85 (m, 2H), 7.43-7.56 (m, 3H), 7.01-7.11 (m, 3H), 6.59 (d, *J* = 4.2 Hz, 1H), 3.64-3.72 (m, 4H), 3.45-3.53 (m, 4H), 2.89 (t, *J* = 6.9 Hz, 2H), 2.41 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.8, 151.3, 147.5, 142.6, 136.1, 134.7, 130.1, 128.7, 123.6, 118.2, 111.0, 108.4, 100.7, 97.6, 66.3, 58.4, 47.9, 41.5.

N-Ethylpyrazolo[1,5-a]pyrimidin-7-amine (2.36a)

To a solution of **2.26** (154 mg, 1.0 mmol) in 5 mL of EtOH under argon was added ethylamine (70% aqueous) (104 μL, 1.3 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.36a** in 68% yield as pale yellow solid. Data for **2.36a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, *J* = 5.4 Hz, 1H), 8.17 (d, *J* = 2.2 Hz, 1H), 7.97 (t, *J* = 6.3 Hz, NH), 6.53 (d, *J* = 2.1 Hz, 1H), 6.21 (d, *J* = 5.4 Hz, 1H), 3.50-3.54 (m, 2H), 1.19 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 150.6, 142.3, 141.8, 139.7, 91.1, 82.0, 37.9, 14.4.

7-Morpholinopyrazolo[1,5-a]pyrimidine (2.36b)

To a solution of **2.26** (154 mg, 1.0 mmol) in 5 mL of EtOH under argon was added morpholine (113 μL, 1.3 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.36b** in 65% yield as beige solid. Data for **2.36b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (d, *J* = 5.1 Hz, 1H), 8.14 (d, *J* = 2.3 Hz, 1H), 6.57 (d, *J* = 2.3 Hz, 1H), 6.37 (d, *J* = 5.0 Hz, 1H), 3.80-3.83 (m, 4H), 3.71-3.74 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 150.8, 150.0, 149.8, 143.4, 95.2, 92.7, 65.7, 48.0.

General procedure for the preparation of 3-nitropyrazolo[1,5-a]pyrimidine derivatives 2.37:

To a solution of **2.36** (1.0 mmol) in 3 mL of H_2SO_4 at 0 °C was added dropwise 1 mL of nitric acid. The reaction mixture was stirred for 30 minutes and 15 mL of water was carefully added to the reaction mixture and small portions of K_2CO_3 were added while stirring until basic pH was reached. An extraction with 2x15 mL of AcOEt was performed. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The product was directly used for the next step without further purification.

N-Ethyl-3-nitropyrazolo[1,5-a]pyrimidin-7-amine (2.37a)

Yellow solid, 50% yield; Data for **2.37a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.37 (d, *J* = 5.3 Hz, 1H), 8.09 (t, *J* = 5.9 Hz, NH), 6.32 (d, *J* = 5.4 Hz, 1H), 3.59-3.63 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 148.7, 139.1, 140.9, 137.2, 95.0, 87.4, 38.3, 14.6.

7-Morpholino-3-nitropyrazolo[1,5-a]pyrimidine (2.37b)

Orange solid, 57% yield; Data for **2.37b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.59 (d, *J* = 5.5 Hz, 1H), 6.42 (d, *J* = 5.5 Hz, 1H), 3.95-3.98 (m, 4H), 3.89-3.92 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.5, 150.5, 145.4, 141.4, 96.1, 66.1, 49.0.

N-(4-fluorobenzyl)pyrazolo[1,5-a]pyrimidin-7-amine (2.36c)

To a solution of **2.26** (154 mg, 1.0 mmol) in 5 mL of EtOH under argon was added *p*-fluoro benzylamine (149 μL, 1.3 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.36c** in 58% yield as beige solid. Data for **2.36c**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (t, *J* = 6.5 Hz, 1H), 8.10 (d, *J* = 2.3 Hz, 1H), 8.08 (d, *J* = 5.2 Hz, 1H), 7.44-7.47 (m, 2H), 7.14-7.17 (m, 2H), 6.42 (d, *J* = 2.3 Hz, 1H), 6.08 (d, *J* = 5.3 Hz, 1H), 4.60 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.2, 160.6, 149.6, 149.1, 146.8, 143.4, 134.4, 129.2, 115.3, 94.7, 85.5, 43.9.

General procedure for the preparation of 2.38 derivatives:

To a solution of **2.37** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of CH_2Cl_2 under argon was added an acid chloride (1.3 mmol) followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.38**.

General procedure for the preparation of 2.39 derivatives:

To a solution of **2.37** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and

concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of CH_2Cl_2 under argon was added an sulfonyl chloride (1.3 mmol) followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.39**.

General procedure for the preparation of 2.40 derivatives:

To a solution of **2.37** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of CH_2Cl_2 under argon was added an isocyanate (1.3 mmol) at room temperature. The reaction mixture was stirred for 2 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.40**.

General procedure for the preparation of 2.41 derivatives:

To a solution of **2.37** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of CH_2Cl_2 under argon was added a chloroformate (1.3 mmol) followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.41**.

General procedure for the preparation of 2.42 derivatives:

To a solution of **2.37** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of EtOH under argon was added an aldehyde (1.3 mmol) followed by sodium cyanoborohydride (126 mg, 2.0 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.42**.

N-(7-(ethylamino)pyrazolo[1,5-a]pyrimidin-3-yl)acetamide (2.38a)

Orange solid, 32% yield; Data for **2.38a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.90 (br. s, NH), 8.41 (s, 1H), 8.10 (d, *J* = 5.2 Hz, 1H), 8.03 (t, *J* = 5.9 Hz, NH), 6.12 (d, *J* = 5.2 Hz, 1H), 3.39-3.44 (m, 2H), 2.06 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 151.7, 149.8, 143.1, 139.7, 112.7, 88.0, 39.6, 26.2, 17.5.

N7-ethyl-N3-tosylpyrazolo[1,5-a]pyrimidine-3,7-diamine (2.39a)

Brown solid, 27% yield; Data for **2.39a**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.71 (br. s, 1H), 8.00-8.02 (m, 2H), 7.74 (s, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.10 (d, *J* = 5.3 Hz, 1H), 3.36-3.40 (m, 2H), 2.33 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.6, 146.6, 143.7, 142.6, 140.5, 137.8, 129.4, 126.9, 105.8, 85.3, 36.3, 21.1, 14.1.

Ethyl 7-morpholinopyrazolo[1,5-a]pyrimidin-3-ylcarbamate (2.41a)

Beige solid, 21% yield; Data for **2.41a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.24 (d, *J* = 5.0 Hz, 1H), 6.36 (d, *J* = 5.0 Hz, 1H), 4.09-4.11 (m, 2H), 3.80-3.82 (m, 4H), 3.72-3.74 (m, 4H), 1.22-1.24 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.3, 149.7, 148.8, 138.4, 92.6, 65.7, 60.3, 47.9, 14.7.

N-(7-morpholinopyrazolo[1,5-a]pyrimidin-3-yl)benzamide (2.38c)

Brown solid, 31% yield; Data for **2.38c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.18 (br. s, NH), 8.46 (s, 1H), 8.29 (d, *J* = 5.0 Hz, 1H), 8.03 (d, *J* = 7.1 Hz, 2H), 7.58 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.2 Hz, 2H), 6.41 (d, *J* = 5.1 Hz, 1H), 3.81-3.84 (m, 4H), 3.75-3.78 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 149.8, 149.0, 143.1, 138.7, 134.1, 131.6, 128.5, 127.8, 108.9, 92.8, 65.7, 48.0.

1-Isopropyl-3-(7-morpholinopyrazolo[1,5-a]pyrimidin-3-yl)urea (2.40b)

Brown solid, 26% yield; Data for **2.40b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.15-8.16 (m, 1H), 7.89 (br. s, NH), 6.25-6.26 (m, 1H), 6.19-6.21 (m, 1H), 3.81-3.82 (m, 4H), 3.75-3.76 (m, 4H), 3.65-3.67 (m, 1H), 1.10-1.11 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.1, 154.8, 149.4, 147.3, 134.5, 111.6, 92.0, 65.7, 47.8, 41.1, 23.2.

General procedure for the preparation of 2.43 derivatives:

To a solution of **2.36** (1.0 mmol) in 5 mL of AcOH under argon was added formaldehyde (37% aqueous solution) (97 μL, 1.3 mmol) followed by an amine (1.3 mmol) at room temperature. The reaction mixture was heated at 65 °C for 16 hours, after completion of the reaction the reaction was allowed to col to room temperature. 15 mL of water was carefully added to the reaction mixture and small portions of K_2CO_3 were added while stirring until basic pH was reached. An extraction with 2x15 mL of AcOEt was performed. The organic layer was dried over Na_2SO_4 and concentrated in vacuo.. The product was purified by preparative HPLC to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.43**.

N-((7-morpholinopyrazolo[1,5-a]pyrimidin-3-yl)methyl)benzenamine (2.43a)

Beige solid, 35% yield; Data for **2.43a**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.26 (d, *J* = 4.9 Hz, 1H), 7.94 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.44-6.46 (m, 3H), 6.33 (d, *J* = 5.0 Hz, 1H), 4.80-4.81 (m, 1H), 3.85 (s, 2H), 3.79-3.81 (m, 4H), 3.70-3.71 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 153.2, 152.2, 151.3, 150.0, 146.2, 132.4, 132.1, 131.9, 117.4, 112.4, 95.7, 69.1, 51.2, 31.1.

N-(4-fluorobenzyl)-3-((cyclohexylamino)methyl)pyrazolo[1,5-a]pyrimidin-7-amine (2.43c)

Brown solid, 33% yield; Data for **2.43c**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (br. s, NH), 8.07 (s, 1H), 8.05 (d, *J* = 5.2 Hz, 1H), 7.43-7.46 (m, 2H), 7.15 (t, *J* = 8.8 Hz, 2H), 6.05 (d, *J* = 5.2 Hz, 1H), 4.58-4.59 (m, 2H), 3.84 (s, 2H), 2.38-2.43 (m, 1H), 1.84-1.85 (m, 2H), 1.63-1.66 (m, 2H), 1.51-1.53 (m, 1H), 1.00-1.20 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.3, 160.6, 148.9, 146.8, 143.0, 134.5, 129.3, 115.3, 107.5, 85.3, 55.2, 43.9, 32.7, 26.0, 24.5.

2-(4-((7-(4-fluorobenzylamino)pyrazolo[1,5-a]pyrimidin-3-yl)methyl)piperazin-1-yl)-N-(pyridin-3-yl)acetamide (2.43d)

Brown solid, 23% yield; Data for **2.43d**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (br. s, NH), 8.75-8.76 (m, 1H), 8.65 (t, *J* = 6.6 Hz, NH), 8.25-8.26 (m, 1H), 8.07 (d, *J* = 5.2 Hz, 1H), 8.03-8.05 (m, 2H), 7.44-7.47 (m, 2H), 7.31-7.33 (m, 1H), 7.16 (t, *J* = 8.8 Hz, 2H), 6.06 (d, *J* = 5.3 Hz, 1H), 4.59 (d, *J* = 6.5 Hz, 2H), 3.64 (s, 2H), 3.11 (s, 2H), 2.43-2.54 (m, 8H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.1, 162.3, 160.7, 149.3, 147.5, 146.8, 144.4, 141.5, 135.3, 134.5, 129.3, 126.8, 123.6, 115.3, 104.1, 85.4, 61.9, 52.9, 52.0, 50.1, 43.9.

4-fluoro-5-phenyl-1H-pyrazol-3-amine (2.50)

A 1M solution of LiHMDS in THF (29.4 mL, 29.4 mmol, 2.0 equiv) was added dropwise to a solution of benzoyl chloride (1.7 mL, 14.68 mmol, 1.0 equiv) and fluoroacetonitrile **2.48** (0.82 mL, 14.68 mmol, 1.0 equiv) in dry THF (15 mL) cooled to -78 °C under nitrogen. The mixture was allowed to reach room temperature after 5h of reaction and quenched with a saturated solution of ammonium chloride. The mixture was concentrated under reduced pressure to afford the desired α-fluoro-β-ketonitrile **2.49** in a form pure enough for the next step.

Hydrazine hydrate (64% in water) (1.4 mL, 29.4 mmol, 2.0 equiv) was added to a solution of the α-fluoro-β-ketonitrile **2.49** (14.68 mmol) in ethanol (20 mL), and the reaction was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with water. The organic phase was concentrated to give a crude product, flash column chromatography on silica gel (ethyl acetate/hexane 90:10) afforded the desired product **2.50** in 50% yield as beige solid. Data for **2.50**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.92 (br. s, 1H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H), 4.77 (br. s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 143.3, 135.9, 134.3, 129.0, 127.9, 126.2, 124.7; ^{19}F NMR (470 MHz, DMSO-d_6) δ –186.5.

Ethyl 3-fluoro-7-hydroxy-2-phenylpyrazolo[1,5-a]pyrimidine-6-carboxylate (2.51)

To a solution of **2.50** (177 mg, 1.0 mmol) in 25 mL of acetic acid under argon at room temperature was added diethyl ethoxymethylenemalonate **2.23** (220 mg, 1.1 mmol). The mixture was heated at 100 °C for 10 hours. The suspension was filtered warm, then washed several times with EtOH, and dried to yield **2.51** in 57% yield as beige solid. Data for **2.51**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.93 (d, *J*=7.4 Hz, 2H), 7.46-7.58 (m, 3H), 4.25 (q, *J*=7.1 Hz, 2H), 1.30 (t, *J*=7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.4, 152.4, 145.4, 140.3, 131.8, 129.8, 129.7, 129.6, 129.2, 128.5, 128.3, 126.6, 98.9, 60.2, 14.4.

3-Fluoro-7-hydroxy-2-phenylpyrazolo[1,5-a]pyrimidine-6-carboxylic acid (2.52)

To a suspension of **2.51** (301 mg, 1.0 mmol) in 30 mL of ethanol and water (1/1) under argon at room temperature was added 2.5 N NaOH (10 mL). The resulting suspension was heated to 85 °C for 3 hours. The suspension was cooled to room temperature and diluted with water (100 mL) and concentrated hydrochlorhydric acid until the formation of a suspension (pH=1). The white suspension was stirred an additional 30 min, filtered, washed with water, and dried to yield **2.52** in 86% yield as white solid. Data for **2.52**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.96 (d, *J*=7.5 Hz, 2H), 7.54 (t, *J*=7.1 Hz, 2H), 7.45 (t, *J*=7.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.0, 159.3, 150.6, 140.1, 135.1, 133.1, 130.7, 129.1, 129.0, 126.5, 95.2.

3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidin-7-ol (2.53)

To the carboxylic acid **2.52** (1 g, 3.66 mmol) was added 10 mL of Dowtherm A. The suspension was heated to 230 °C for 5h. The reaction mixture was cooled to room temperature, then diluted with 30 mL of hexanes, stirred vigorously, filtered and dried to obtain the desired product **2.53** in 82% yield as light brown solid. Data for **2.53**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (d, *J* = 7.6 Hz, 2H), 7.75 (d, *J* = 5.9 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 1H), 5.48 (d, *J* = 5.9 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.2, 148.0, 136.9, 131.8, 131.7, 128.8, 128.3, 126.5, 92.8.

7-chloro-3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidine (2.54)

2.53 (229 mg, 1.0 mmol) was dissolved in 10 mL of phosphorus oxychloride at room temperature. The reaction mixture was heated at 95 °C for 4 hours after which the color of the solution became brown. The flask was allowed to cool to room temperature and the excess of phosphorus oxychloride was concentrated in vacuo to a brown oil. The oil was dissolved in 15 mL of EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated to afford crude **2.54** as brown oil used directly without further purification.

General procedure for the preparation of pyrazolo[1,5-a]pyrimidine derivatives 2.55:

To a solution of **2.54** (248 mg, 1.0 mmol) in 5 mL of EtOH under argon was added an amine (1.3 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) to afford the amino substituted pyrazolo[1,5-a]pyrimidine **2.55**.

N-ethyl-3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidin-7-amine (2.55a)

White solid, 70% yield; mp 212-216°C; Data for **2.55a**: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.16 (d, *J* = 5.3 Hz, 1H), 8.13 (t, *J* = 5.8 Hz, 1H), 8.04 (d, *J* = 7.3 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 2H), 7.47 (t, *J* = 7.5 Hz, 1H), 6.22 (d, *J* = 5.3 Hz, 1H), 3.48 (m, 2H), 1.26 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d6) δ 149.8, 146.0, 138.7, 136.9, 135.1, 133.5, 130.6, 129.1, 126.7, 85.5, 36.6, 14.2.

3-Fluoro-7-morpholino-2-phenylpyrazolo[1,5-a]pyrimidine (2.55b)

Beige solid, 63% yield; Data for **2.55b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (d, *J* = 5.0 Hz, 1H), 7.99 (d, *J* = 7.5 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.47 (t, *J* = 7.3 Hz, 1H), 6.44 (d, *J* = 5.2 Hz, 1H), 3.82-3.86 (m, 8H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.8, 149.0, 138.5, 138.3, 135.5, 133.9, 130.2, 129.4, 129.2, 93.4, 65.6, 48.1.

***Tert*-butyl 4-(3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidin-7-ylamino)piperidine carboxylate (2.55c)**

Beige solid, 69% yield; Data for **2.55c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 5.1 Hz, 1H), 8.09 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 7.9 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 1H), 6.38 (d, *J* = 8.2 Hz, NH), 5.95 (d, *J* = 5.2 Hz, 1H), 4.13-4.17 (m, 2H), 2.99-3.07 (m, 2H), 2.14-2.19 (m, 2H), 1.61-1.74 (m, 3H), 1.51 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4, 144.2, 139.7, 135.1, 132.0, 131.8, 131.3, 129.3, 125.5, 123.9, 123.6, 121.8, 80.0, 74.9, 44.4, 26.6, 23.3.

3-Fluoro-2-phenyl-N-(piperidin-4-yl)pyrazolo[1,5-a]pyrimidin-7-amine (2.55d)

White solid, 65% yield; Data for **2.55d**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, NH), 7.55 (t, *J* = 7.5 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 6.31 (d, *J* = 5.4 Hz, 1H), 3.69-3.71 (m, 1H), 3.00-3.03 (m, 2H), 2.59-2.64 (m, 2H), 1.87-1.89 (m, 2H), 1.61-1.69 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.8, 145.2, 138.7, 137.1, 136.9, 135.3, 133.3, 130.5, 129.2, 129.0, 126.8, 126.7, 86.0, 50.0, 45.05, 32.4.

3-Fluoro-N-(1-(methylsulfonyl)piperidin-4-yl)-2-phenylpyrazolo[1,5-a]pyrimidin-7-amine (2.55e)

White solid, 60% yield; Data for **2.55e**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 7.4 Hz, 2H), 7.91 (d, *J* = 8.9 Hz, NH), 7.56 (t, *J* = 7.8 Hz, 2H), 7.47 (t, *J* = 7.3 Hz, 1H), 6.36 (d, *J* = 5.3 Hz, 1H), 3.81-3.88 (m, 1H), 3.65-3.68 (m, 2H), 2.91-2.93 (m, 5H), 2.02-2.03 (m, 2H), 1.84-1.90 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.8, 145.4, 138.9, 135.1, 133.5, 130.5, 129.2, 129.0, 126.8, 86.1, 48.6, 44.8, 34.8, 30.4.

1-(4-(3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidin-7-ylamino)piperidin-1-yl)ethanone (2.55f)

White solid, 58% yield; Data for **2.55f**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (d, *J* = 5.1 Hz, 1H), 8.07 (d, *J* = 7.5 Hz, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 1H), 6.38 (d, *J* = 8.2 Hz, NH), 5.95 (d, *J* = 5.2 Hz, 1H), 4.59-4.62 (m, 1H), 3.91-3.94 (m, 1H), 3.77-3.83 (m, 1H), 3.28-3.33 (m, 1H), 2.92-2.96 (m, 1H), 2.19-2.24 (m, 2H), 2.16 (s, 3H), 1.66-1.74 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.0, 149.3, 144.7, 140.4, 137.0, 136.9, 136.2, 134.6, 130.5, 129.1, 128.8, 126.9, 85.2, 49.4, 44.8, 40.0, 32.3, 31.5, 21.5.

N-benzyl-3-fluoro-2-phenylpyrazolo[1,5-a]pyrimidin-7-amine (2.55g)

Beige solid, 68% yield; Data for **2.55g**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (t, *J* = 6.5 Hz, NH), 8.10 (d, *J* = 5.2 Hz, 1H), 8.05 (d, *J* = 7.3 Hz, 2H), 7.57 (t, *J* = 7.5 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 6.14 (d, *J* = 5.3 Hz, 1H), 4.68 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.7, 146.2, 138.9, 138.0, 137.0, 136.8, 135.2, 133.6, 130.6, 129.2, 129.0, 128.6, 127.4, 127.2, 126.7, 86.2, 44.7.

3-Fluoro-2-phenyl-N-((pyridin-3-yl)methyl)pyrazolo[1,5-a]pyrimidin-7-amine (2.55h)

Beige solid, 58% yield; Data for **2.55h**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.62 (d, *J* = 4.7 Hz, 1H), 8.19 (d, *J* = 5.0 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 2H),

7.42 (t, *J* = 7.2 Hz, 1H), 7.33-7.35 (m, 1H), 6.90 (t, *J* = 5.6 Hz, NH), 5.92 (d, *J* = 5.2 Hz, 1H), 4.68 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.8, 149.5, 148.9, 145.7, 140.5, 136.9, 136.8, 136.4, 134.9, 134.7, 131.7, 130.5, 129.1, 128.8, 126.9, 123.9, 85.5, 43.7.

3-Fluoro-2-phenyl-N-(1H-pyrazol-3-yl)pyrazolo[1,5-a]pyrimidin-7-amine (2.55i)

White solid, 55% yield; Data for **2.55i**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.10 (br. s, NH), 10.25 (s, 1H), 8.31 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 7.6 Hz, 2H), 7.78-7.79 (s, 1H), 7.59 (t, *J* = 7.6 Hz, 2H), 7.50 (t, *J* = 7.2 Hz, 1H), 7.33 (d, *J* = 5.2 Hz, 1H), 6.39-6.40 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 150.3, 147.1, 142.6, 138.8, 137.1, 136.9, 135.4, 133.8, 130.5, 129.6, 129.3, 129.0, 126.8, 97.2, 89.9.

General procedure for the preparation of pyrazolo[1,5-a]pyrimidine derivatives 2.58:

To a solution of **2.50** (150 mg, 0.85 mmol) in 5 mL of AcOH under argon was added a (hetero)aryl dimethylamino propenone (1.3 mmol) at room temperature. The reaction mixture was refluxed for 16 hours. The suspension was filtered warm, then washed several times with ethanol and dried to obtain the desired product **2.58**.

3-Fluoro-2,7-diphenylpyrazolo[1,5-a]pyrimidine (2.58a)

Beige solid, 50% yield; Data for **2.58a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.63 (d, *J* = 4.4 Hz, 1H), 8.19-8.22 (m, 2H), 7.98 (d, *J* = 7.3 Hz, 2H), 7.65-7.67 (m, 3H), 7.55-7.58 (m, 2H), 7.47-7.50 (m, 1H), 7.30 (d, *J* = 4.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.8, 145.1, 139.5, 137.2, 136.3, 134.3, 131.4, 130.1, 130.0, 129.7, 129.6, 129.5, 129.2, 128.7, 126.7, 108.6.

3-Fluoro-2-phenyl-7-(thiophen-2-yl)pyrazolo[1,5-a]pyrimidine (2.58b)

Yellow solid, 42% yield; mp 233-237°C; Data for **2.58b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (d, *J* = 4.6 Hz, 1H), 8.59 (d, *J* = 3.8 Hz, 1H), 8.18 (d, *J* = 5.0 Hz, 1H), 8.13 (d, *J* = 7.7 Hz, 2H), 7.82 (d, *J* = 4.6 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 2H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 4.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.0, 139.2, 138.6, 136.2, 135.0, 134.2, 132.5, 130.0, 129.6, 129.3, 129.2, 127.8, 126.6, 104.6.

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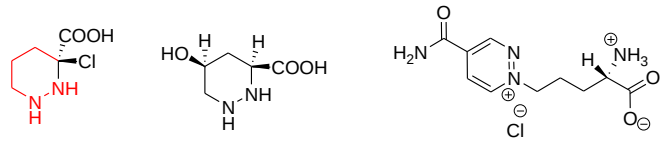
Chapter III. Synthesis and biological evaluation of substituted pyridazine derivatives and novel fused pyridazine analogues

1. Introduction

The first synthesis of the pyridazine was described in 1895 by Taüber[1] but was not considered as relevant by the medicinal chemistry community compared to other diazines such as pyrimidines and pyrazines. The interest raised from 1971 with the discovery of the naturally occurring hexahydropyridazine amino acids **3.1** reported by Hassall *et al.*[2] followed in 1988 by the first natural product containing a pyridazine, pyridazomycin **3.2**.[3] After these discoveries, more attention has been directed towards the pyridazine ring and its application in medicinal chemistry.



3.1 **Hexahydropyridazine amino acids**

3.2 **Pyridazomycin**

Figure 1. Naturally occurring pyridazine derivatives.

Recently, pyridazines have been considered by GlaxoSmithKline as one of the "most developable" heteroaromatic rings for drug design.[4] Pyridazine analogues proved to be useful ligands for different targets and have been proposed as "privileged structure" for drug discovery.[5] Several compounds with pyridazine rings demonstrate biological activity and many examples of pyridazine structures are naturally occurring.[6] Pyridazines were recognized as selective GABA-A receptor antagonists, such as minaprine **3.3**.[7] Volonterio *et al.* develop the synthesis of pyridazine-based scaffolds such as **3.4** to target protein/protein interaction as α-helix mimetics.[8] 3-Amino-6-aryl-pyridazines have been considered as an interesting pharmacophore in drug discovery. Some compounds show biological activitiy ranging from obesity[9] or neurodegenerative diseases[10] to inflammatory pain such as the selective CB_2 agonist **3.5**.[11] Among kinase inhibitors, different compounds containing the diazine pyridazine have been identified. For example, compound **3.6** is based on a pyridazinone ring and has been identified as a potent p38 MAP inhibitor[12] (Figure 1).

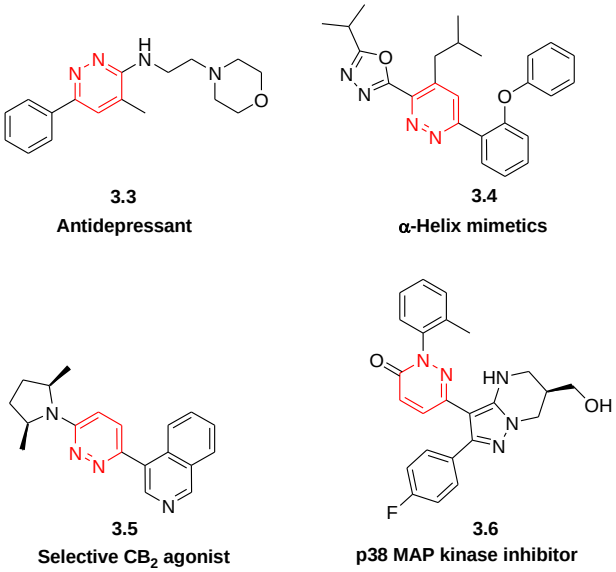
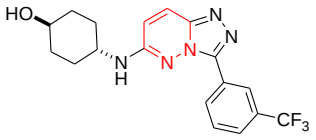


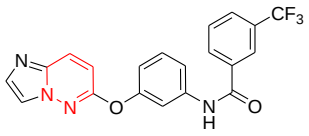
Figure 2. Biologically active pyridazine derivatives.

The nitrogen containing heterocycle pyridazine is a key intermediate in the synthesis of several fused heterocycles used in drug discovery.[13] Fused pyridazines can be distinguished into two classes. The first one having a bridgehead nitrogen atom. Some of them can be obtained from substituted 3-amino-pyridazine which is easily available from substituted 3-chloro-pyridazine, and further cyclized to a bi-heterocyclic structure. Generally rings fused to pyridazines are 5-membered rings. Triazolo[4,3-b]pyridazine such as PIM-1 inhibitor **3.7**[14] can be considered as an example of this class of fused pyridazines. These derivatives were obtained after hydrazinolysis and cyclization of substituted 3-chloro-pyridazines. Another example of this class of compounds is a VEGFR2 kinase inhibitor based on a imidazo[1,2-b]pyridazine **3.8** scaffold,[15] which can be obtained by condensing substituted 3-amino-pyridazines with 2-chloro-acetaldehyde. The second class represents pyridazines fused to a 6- or 5-membered ring, without bridgehead nitrogen atom. Examples of this class of compounds are the phosphodiesterase 10A inhibitor **3.9**[16] and the melanin-concentrating hormone 1 antagonist[17] **3.10** (Figure 2).

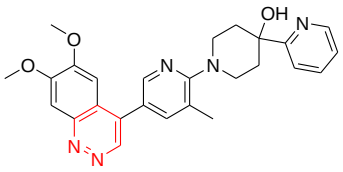
A strategy for the synthesis of functionalized pyridazines has been developed, a versatile building block for further modification and especially on the position 4 of the ring. In order to extend the scope of this process, many efforts have been made to synthesize different novel fused pyridazines.



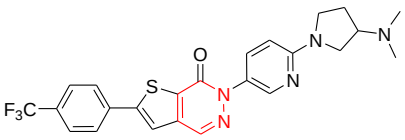
3.7
Pim-1 kinase inhibitor



3.8
VEGFR2 kinase inhibitor



3.9
PDE10A inhibitor

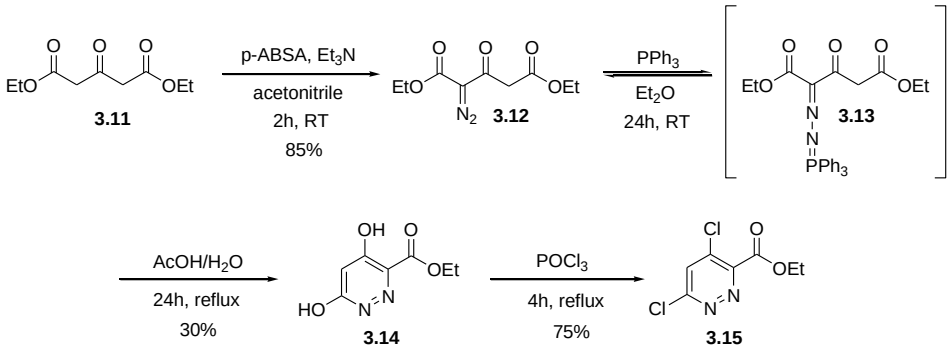


3.10
Melanin-concentrating hormone receptor 1 antagonist

Figure 3. Biologically active fused pyridazine derivatives.

2. Results and discussion

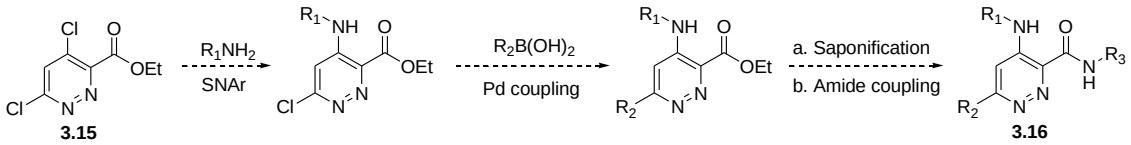
The development of a library of pyridazine derivatives has been envisioned based on the methodology described by Neurogen in 2004.[18] The starting material was the diethyl acetone dicarboxylate **3.11**, which was subjected to a diazo transfer reagent to obtain **3.12**. The diazo derivative **3.12** was then converted to a phosphazine **3.13** with triphenyl phosphine (equilibrium) and directly heated in a mixture of acetic acid and water at 105°C to afford the desired pyridazine **3.14** in moderate yield over two steps. A final chlorination with $POCl_3$ led to the key intermediate **3.15**, the pyridazine obtained can be modified in a site selective way, in fact three points of substitution became available, the ester group at position 3 and the two chlorides at position 4 and position 6.



Scheme 1. Synthesis of 4,6-dichloro-3-ethoxycarbonyl pyridazine.

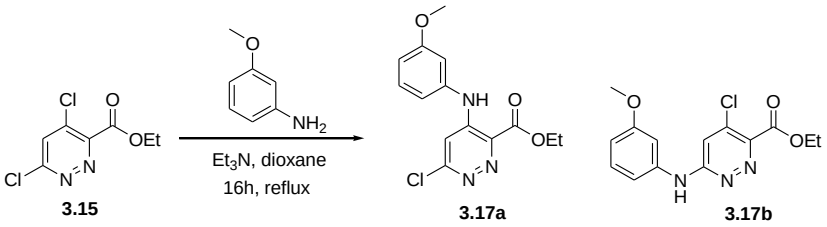
Some examples in literature have shown that the reactivity of the position 4 towards nucleophilic substitution is favorised compared to the position 6,[19] thus a nucleophilic substitution has been chosen to be the first step, followed by a Suzuki palladium cross-

coupling at the position 6 and finally the formation of an amide to get the desired product **3.16**.



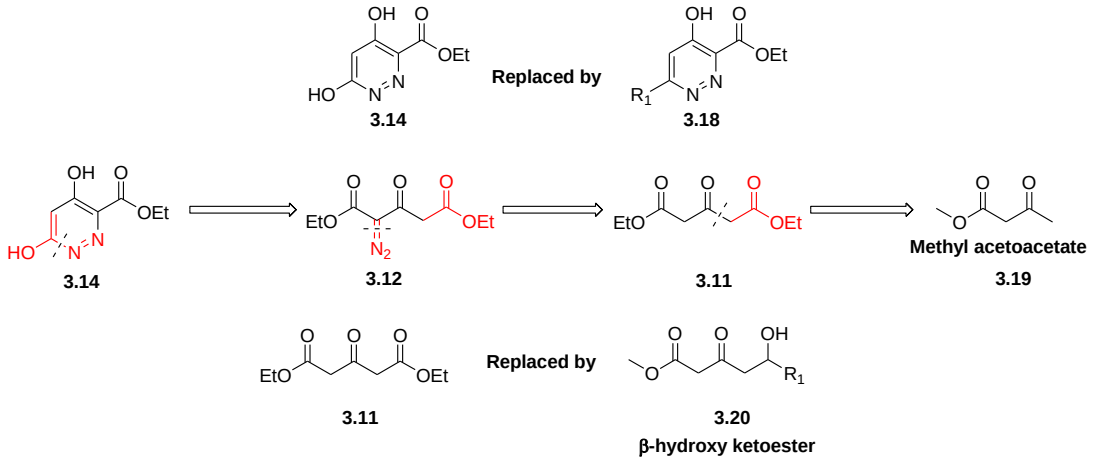
Scheme 2. Synthesis of **3.16** from 4,6-dichloro-3-ethoxycarbonyl pyridazine **3.15**.

A first attempt of nucleophilic substitution with *m*-anisidine has been done but the LC/MS analysis showed a mixture of two products with the same mass, the two isomers have been synthesized without selectivity. Unfortunately, no isolation of **3.17a** and **3.17b** was possible.



Scheme 3. Nucleophilic substitution with *m*-anisidine.

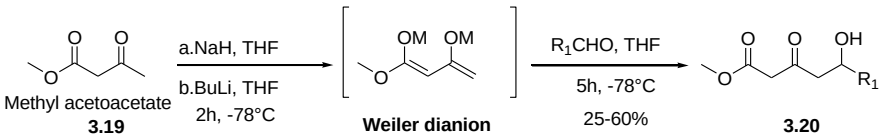
From this observation, a methodology allowing the introduction of substituents in a site selective way was highly desirable. The synthesis of a pyridazine already substituted at one of the position (4 or 6) could be very interesting for the rest of the synthesis, with a short retrosynthetic study, we can see that it is easier to introduce a variation at the position 6 than 4 because C6 is involved in the final cyclization with the diazo group.



Scheme 4. Retrosynthetic study.

In our search for operationally simple processes, we have investigated a new method to prepare pyridazines under mild conditions. It has been decided to replace one of the

ester groups of diethyl acetone dicarboxylate **3.11** by a ketone to facilitate the cyclization reaction. However, the diazo transfer reaction may then occur at two positions. One position can be privileged by the introduction of an alcohol group instead of an ester group or a ketone. Thus, the replacement of **3.14** by a novel intermediate **3.18** could be of high value and in order to synthesize this pyridazine, it was necessary to modify the starting material **3.11**. By using an aldol reaction developed by Weiler[20] based on the dianion of the methyl acetoacetate **3.19**, it is possible to introduce a substituted alcohol instead of an ester group and to obtain a β-hydroxy ketoester **3.20**.



Scheme 5. Weiler dianion chemistry.

The formation of the dianion is generated from the treatment of the methyl acetoacetate with first one equivalent of sodium hydride followed by one equivalent of *n*-BuLi at -78°C, after 1 hour of reaction, an aldehyde was added to afford the β-hydroxy ketoester **3.20**.

Prompted by the need for a more reliable and versatile preparation of pyridazines allowing the introduction of various substituents such as alkyls, cycloalkyls, aryls, and heteroaryls, we envisioned a more convenient synthesis of 6-substituted-4-hydroxy-3-methoxycarbonyl pyridazines starting from methyl acetoacetate **3.19**. The synthesis of a new pyridazine derivative has been developed in four steps with moderate to good yields. To the best of our knowledge, the only existing procedure leading to 3-alkoxycarbonyl-4-hydroxy-6-substituted pyridazines **3.18** is the one developed by Zelesov *et al*. starting from furan-4,5-dione.[21] However, this method is limited to the synthesis of aryl substituted compounds at the C6 position, such as compound **3.21**. Importantly, and of particular value for the development of a versatile method for the synthesis of pyridazine derivatives, is that the obtained compounds can be further modified in a site-selective way. For example, selective reaction of 4,6-dichloro pyridazine **3.15** at positions 4 and 6 was problematic as previously discussed.

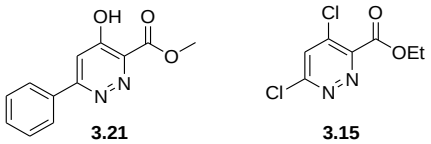
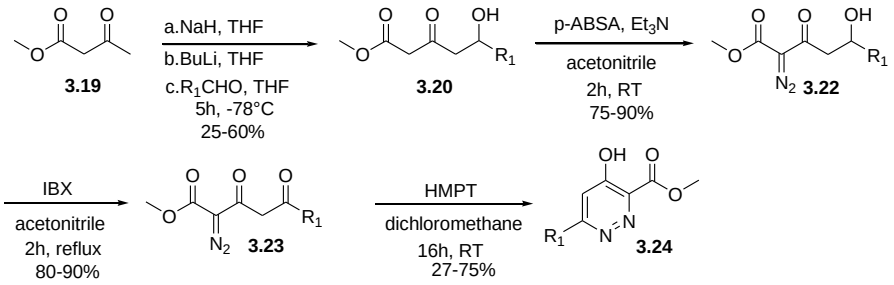


Figure 4. Pyridazine derivatives.

The synthesis starts with the reaction of Weiler dianion[22] of methyl acetoacetate **3.19** with an aldehyde, which led to the β-hydroxy ketoester **3.20** in moderate yields. These aldol products are easily degraded (water elimination) to give the corresponding enone. After treatment of **3.20** with *p*-ABSA (p-acetamido benzene sulfonyl azide) in acetonitrile, the diazo derivative **3.22** was obtained in good yields.[23] A mild oxidation of **3.22** using IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide)[24] in acetonitrile for 2h under reflux led to the corresponding α-diazo-β-ketoester **3.23** in good yields. The final stages of the synthesis (the formation of phosphazine and the subsequent diaza-Wittig reaction) occur as a tandem process to obtain the pyridazine **3.24** in moderate to good yields.

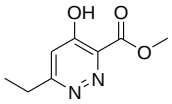
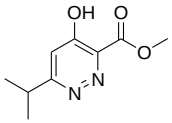
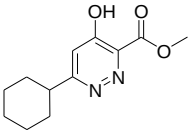
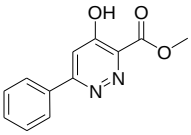
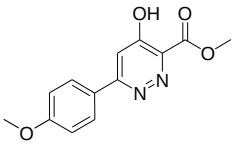
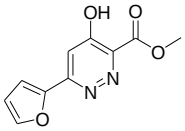
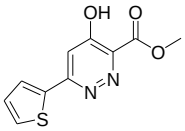
This procedure allowed the introduction of different groups at the C6 position of the pyridazine ring, such as alkyls, cycloalkyls, aryls and heteroaryls by selecting the appropriate aldehyde as starting material. In this respect this procedure proved to be more selective than a strategy based on palladium cross-coupling.



Scheme 6. Synthesis of 6-substituted-4-hydroxy-3-methoxycarbonyl pyridazines.

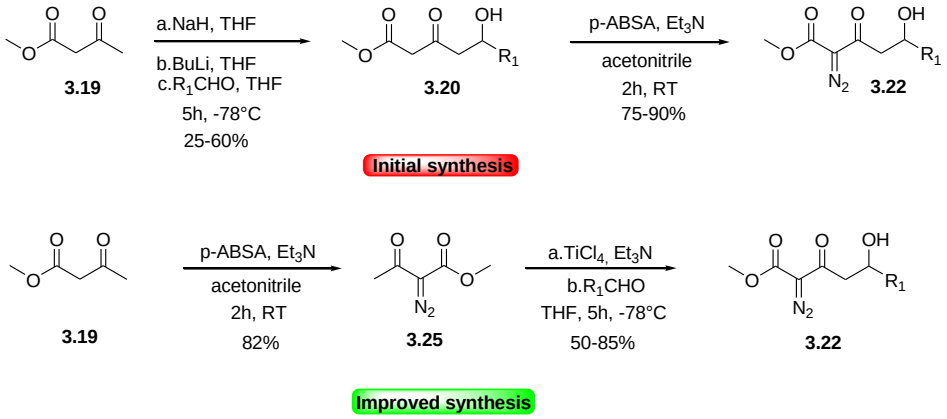
We observed that better yields were obtained with alkyl aldehydes or cycloalkyl aldehydes than with aryl aldehydes and heteroaryl aldehydes. In order to cover a wide variety of substitutions, different attempts using aryl aldehydes containing electron withdrawing groups like *p*-nitrobenzaldehyde have been explored but did not give the required β-hydroxy ketoester **3.22** in our hands.

Table 1. Synthesis of 6-substituted-4-hydroxy-3-methoxycarbonyl pyridazines.[a]

entry	aldehyde derivative	pyridazine		yield of **3.24** (%)[b]
1	propionaldehyde		**3.24a**	68
2	isobutyraldehyde		**3.24b**	75
3	cyclohexane carboxaldehyde		**3.24c**	62
4	benzaldehyde		**3.24d**	60
5	*p*-anisaldehyde		**3.24e**	45
6	2-furfural		**3.24f**	27
7	thiophene-2-carboxaldehyde		**3.24g**	33

[a]All reactions were performed using 1 mmol of **3.22** and 364 mg of IBX in refluxing acetonitrile for 2 hours, followed by a filtration to obtain the corresponding α-diazo-β-ketoester. The crude compound was stirred with 183 μL of HMPT in dichloromethane for 16 hours. [b]Isolated yield.

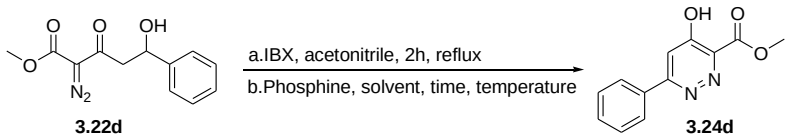
Due to the low yields of the first step of the procedure and the toxicity of the HMPT[25] used for the Diaza Wittig reaction, an improvement of the method to synthesize versatile pyridazines has been investigated. Calter et al.[26] described an aldol reaction from α-diazo-β-ketoester **3.25** using titanium chloride to afford the diazo derivative **3.22** directly in good yields. In fact, while the diazo transfer reaction was done directly on **3.19** followed by the titanium aldol reaction with appropriate aldehyde, **3.22** was obtained in better yields than the initial method.



Scheme 7. Synthesis of **3.22** with the Calter's method.

The replacement of the HMPT by a safer reagent was then the most important modification to do, different phosphines have been screened to convert the derivative **3.22d** into the pyridazine **3.24d** after oxidation with IBX. A first attempt to replace HMPT by triphenyl phosphine was not successful. In fact, after two days of reaction in diethyl ether at room temperature, no α-diazo-β-ketoester **3.23d** was converted into the corresponding pyridazine (Table 2, entry 2). An increase of the temperature to 60°C by using tetrahydrofuran as a solvent led to the same result (Table 2, entry 3). By using triethyl phosphite, the pyridazine was obtained in a lower yield than with HMPT (Table 2, entry 4). Triethyl phosphine allowed the formation of the pyridazine in 5 hours in dichloromethane (Table 2, entry 5). Due to the toxicity and facile oxidation of triethyl phosphine,[27] the tributyl phosphine was explored and the pyridazine was obtained in less than 1 hour at room temperature (Table 2, entry 6) in good yields. Diisopropyl ether (*i*-Pr_2O) proved to be the best solvent for this reaction, allowing to obtain the pyridazine **3.24d** as precipitate in less than 30 minutes in high yields (Table 2, entry 8). This modification represents an improved and safe method for the synthesis of the pyridazine derivatives **3.24**.

Table 2. Influence of phosphines and solvents on the Diaza-Wittig reaction.[a]

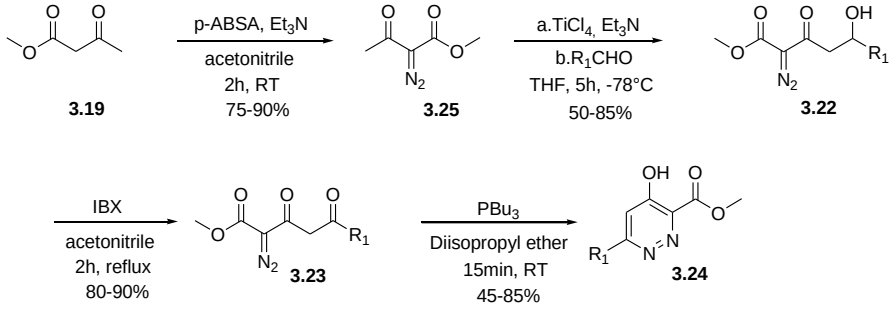


3.22d **3.24d**

entry	phosphine	solvent	conditions	yield of **3.24d** (%)[b]
1	HMPT	CH_2Cl_2	16 h, RT	60 [c]
2	$P(Ph)_3$	Et_2O	48 h, RT	- [d]
3	$P(Ph)_3$	THF	16 h, 60°C	- [d]
4	$P(OEt)_3$	CH_2Cl_2	16 h, RT	37 [c]
5	$P(Et)_3$	CH_2Cl_2	5 h, RT	52 [c]
6	P(*n*-Bu)$_3$	CH_2Cl_2	1 h, RT	58 [c]
7	P(*n*-Bu)$_3$	Et_2O	30 min, RT	53 [c]
8	P(*n*-Bu)$_3$	*i*-Pr_2O	30 min, RT	70 [e]

[a]All reactions were performed using 1 mmol of **3.22d** and 364 mg of IBX in refluxing acetonitrile for 2 hours, followed by a filtration to obtain the α-diazo-β-ketoester. The crude compound was stirred with 183 μL of phosphine following the conditions described above. [b]Isolated yield. [c]Purification by flash column chromatography on silica gel. [d]α-diazo-β-ketoester **3.23d** recovered. [e]Precipitation of **3.24d**.

These two modifications led to an improved and safe method for the synthesis of pyridazine derivatives.

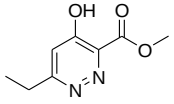
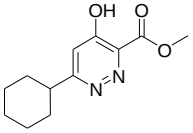
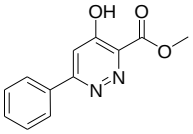
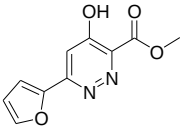


3.19 **3.25** **3.22** **3.23** **3.24**

Scheme 8. Improved method for the synthesis of pyridazine derivatives.

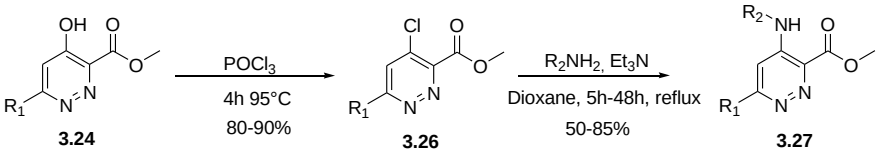
Invariable, the use of P(*n*-Bu)$_3$ gives higher yields of pyridazines than while using HMPT. Most of the derivatives were obtained as precipitate (Table 3, entry 1-3) in less than 30 minutes of reaction.

Table 3. Synthesis of 6-substituted-4-hydroxy-3-methoxycarbonyl pyridazines.[a]

entry	aldehyde derivative	pyridazine		yield of **12** (%)[b] (HMPT method)	yield of **12** (%)[b] (P(*n*-Bu)$_3$ method)
1	propionaldehyde		**3.24a**	68 [c]	85 [d]
2	cyclohexane carboxaldehyde		**3.24c**	62 [c]	72 [d]
3	benzaldehyde		**3.24d**	60 [c]	70 [d]
4	2-furfural		**3.24f**	27 [c]	35 [c]

[a]All reactions were performed using 1 mmol of **3.22** and 364 mg of IBX in refluxing acetonitrile for 2 hours, followed by a filtration to obtain the corresponding α-diazo-β-ketoester. The crude compound was stirred with 183 μL of HMPT in dichloromethane for 16 hours (HMPT method) or with 250 μL of P(*n*-Bu)$_3$ in *i*-Pr$_2$O for 30 minutes (P(*n*-Bu)$_3$ method). [b]Isolated yield. [c]Purification by flash column chromatography on silica gel. [d]Precipitation of **3.24**.

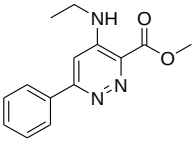
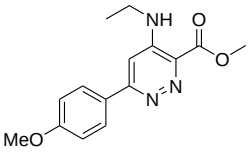
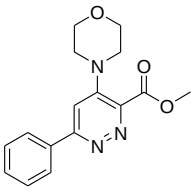
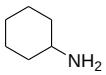
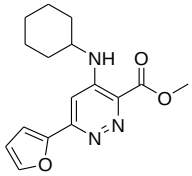
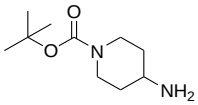
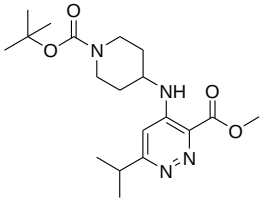
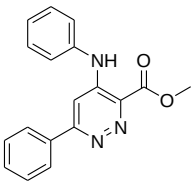
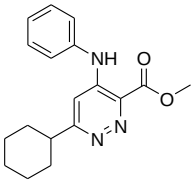
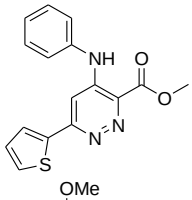
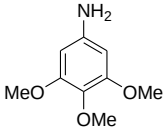
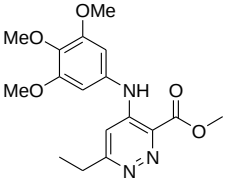
Pyridazine **3.24** was subjected to chlorination to get **3.26** followed by a nucleophilic substitution to afford compound **3.27**. Different amines have been used for the nucleophilic substitution from aliphatic amines and cycloalkyl amines introduced in a couple of hours to aromatic amines which needed more than 16 hours of reaction, both of the introduction have been done in refluxing dioxane with triethylamine as base.

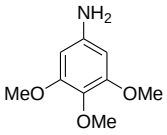


Scheme 9. Nucleophilic substitution on pyridazines.

The yields obtained were better with aliphatic and cyclalkyl amines than aromatic amines.

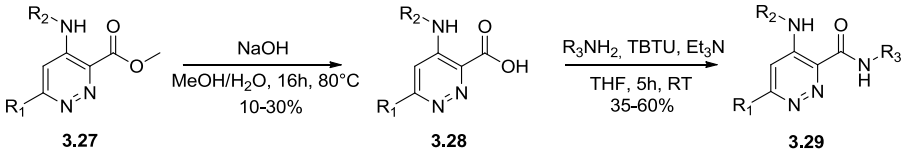
Table 4. Substrate scope of the nucleophilic substitution on pyridazine derivatives.[a]

entry	starting material	amine	pyridazine		yield (%)[b]
1	**3.24d**			**3.27a**	85
2	**3.24**[e]			**3.27b**	83
3	**3.24d**			**3.27c**	83
4	**3.24f**			**3.27d**	75
5	**3.24b**			**3.27e**	68
6	**3.24d**			**3.27f**	52
7	**3.24c**			**3.27g**	57
8	**3.24g**			**3.27h**	50
9	**3.24a**			**3.27i**	62

10	**3.24d**			**3.27j**	56

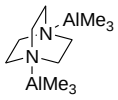
[a]All reactions were performed using 1 mmol of **3.24**, 1.1 mmol of amine and 181 µL of triethylamine in refluxing dioxane until completion of the reaction monitored by TLC. [b]Isolated yield.

The last part of the synthesis was the formation of an amide bond, two different methods have been attempted. First, **3.27** has been converted to the corresponding carboxylic acid **3.28** by saponification and then coupled with various amines with an amide coupling reagent to get **3.29**.



Scheme 10. Saponification and amide coupling.

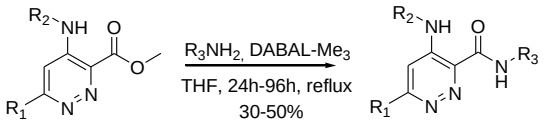
Unfortunately the saponification was low yielding and the work has been directed towards a method converting directly the ester group into amides. The most convenient way was the use of DABAL-Me_3 developed by the Woodward group,[28] this reagent is a safe alternative to the dangerous trimethyl aluminium. DABAL-Me_3 is an adduct of trimethylaluminum and DABCO that is a free-flowing solid that can be manipulated without the need for an inert atmosphere.



DABAL-Me_3

Figure 5. DABAL-Me_3, direct amide formation reagent.

Novak *et al*. described a good yielding direct amide formation[29] from unactivated esters and amines, this method has been applied to derivative **3.27** to obtain amide **3.29**, different amines were used such as aliphatic amines, cycloalkyl amines and (hetero)aromatic amines.

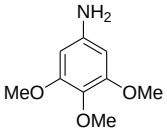
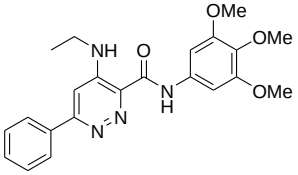
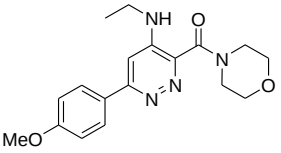
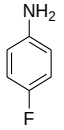
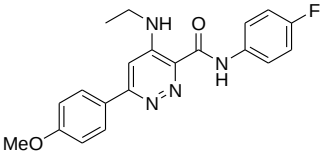
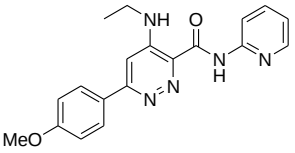
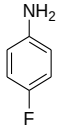
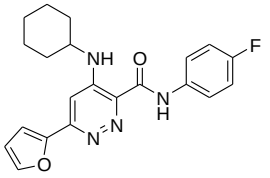
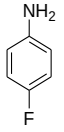
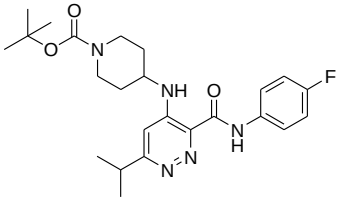
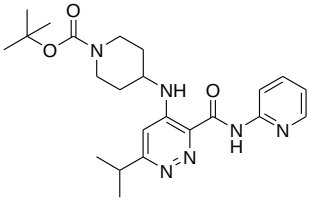


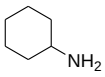
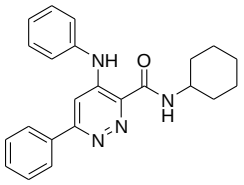
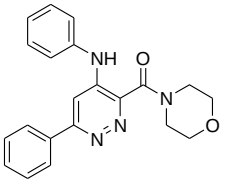
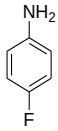
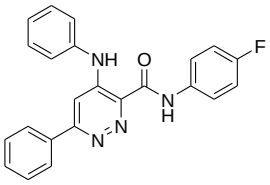
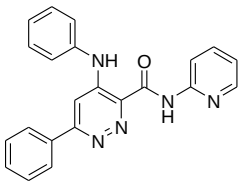
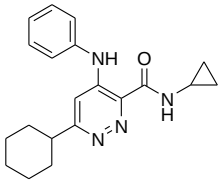
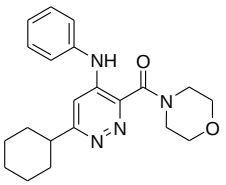
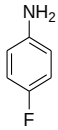
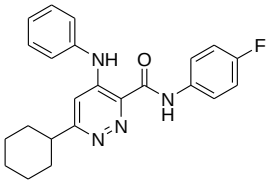
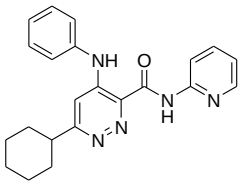
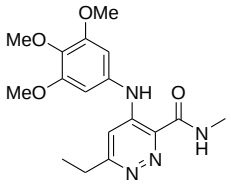
3.27 **3.29**

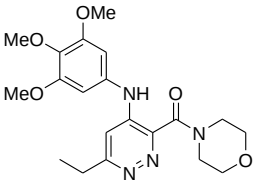
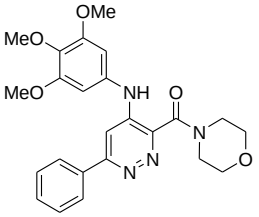
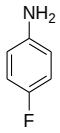
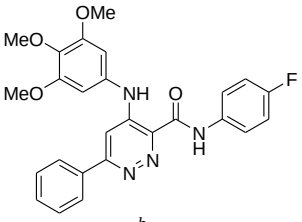
Scheme 11. Amide formation with Dabal-Me_3.

The synthesis of a library of novel pyridazine derivatives **3.29** has been built with three different points of substitution.

Table 5. Substrate scope of the amide formation.[a]

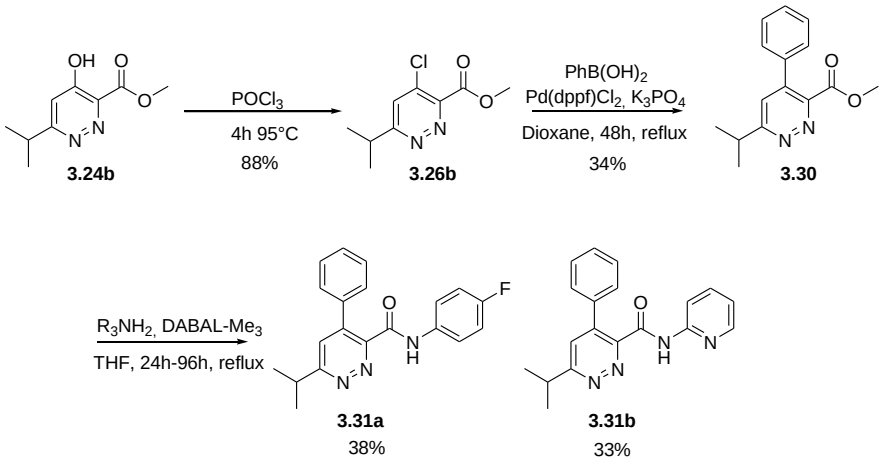
entry	starting material	amine	pyridazine		yield (%)[b]
1[c]	**3.27a**			**3.29a**	37
2[c]	**3.27b**			**3.29b**	43
3[c]	**3.27b**			**3.29c**	38
4[c]	**3.27b**			**3.29d**	32
5[c]	**3.27d**			**3.29e**	35
6[c]	**3.27e**			**3.29f**	41
7[c]	**3.27e**			**3.29g**	30

8[c]	**3.27f**			**3.29h**	47
9[c]	**3.27f**			**3.29i**	50
10[c]	**3.27f**			**3.29j**	47
11[c]	**3.27f**			**3.29k**	33
12[c]	**3.27g**			**3.29l**	48
13[c]	**3.27g**			**3.29m**	44
14[c]	**3.27g**			**3.29n**	39
15[c]	**3.27g**			**3.29o**	31
16[d]	**3.27i**			**3.29p**	60

17[d]	**3.27i**			**3.29q**	56
18[d]	**3.27j**			**3.29r**	51
19[d]	**3.27j**			**3.29s**	35

[a]Two methods have been used for the synthesis of amides. [b]Isolated yield. [c]1.5 mmol of amine and 385 mg of Dabal-Me_3 were stirred at 40 °C for 1 hour, after which 0.5 mmol of **3.27** was added. The reaction mixture was refluxed in THF until completion of the reaction monitored by TLC. [d]To a solution of 0.5 mmol of **3.27** in MeOH/water was added 200 mg of sodium hydroxyde dissolved in water, the reaction was heated at 80 °C for 16 hours. After an acidic workup and evaporation to the carbolylic acid derivative **3.28**, the crude was used directly without further purification. To a solution of **3.28** in THF was added 83 µL of triethylamine, followed by 209 mg of TBTU and 0.75 mmol of amine. The reaction was stirred at room temperature until completion of the reaction monitored by TLC.

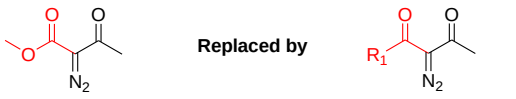
4-aryl pyridazines are known to be biologically active compounds towards different receptors,[30] in order to expand the library, a Suzuki palladium cross-coupling has been done between **3.26b** and phenyl boronic acid. The yield obtained for **3.30** was low and no optimization was attempted, **3.30** was directly used for the formation of amides with Dabal-Me3 to afford **3.31**.



Scheme 12. Synthesis of 4-aryl pyridazine derivatives **3.31**.

Based on this novel methodology developed for the synthesis of pyridazine derivatives bearing an ester group, a different starting material has been chosen to deliver a pyridazine bearing a ketone group, an interesting intermediate to access novel fused pyridazines.

For the building of pyridazines bearing a 3-keto group, starting material of type **3.32** is needed. This approach will, likewise, allow us to obtain pyridazine derivatives, useful for the formation of new bicyclic heterocycles.



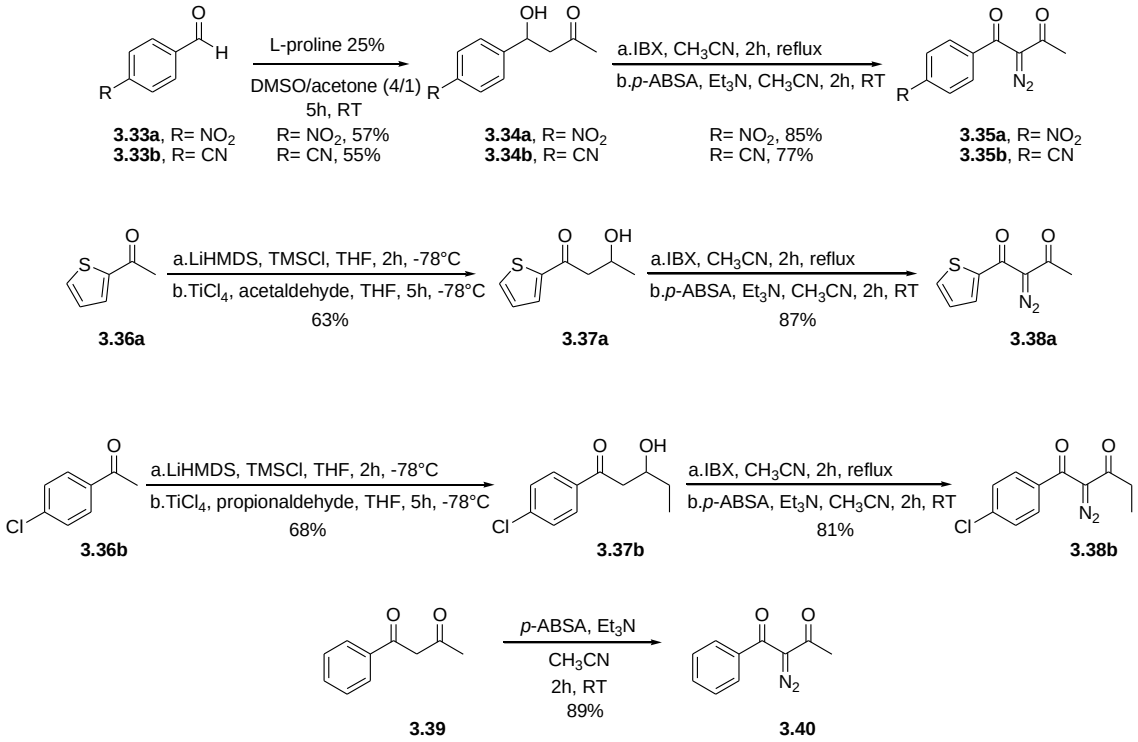
3.25 **3.32**

R_1= aryl, heteroaryl

Figure 6. Diazo derivatives.

The reaction scheme starts with the synthesis of β-hydroxy ketone. In order to avoid any side reaction during the synthetic route, R_1 has been chosen to be an aryl or heteroaryl group. Different methods for the synthesis of 1,3-diketone have been described in the past.[31] We have followed two different approaches. The first one is based on L-proline organocatalysis discovered by List *et al.*.[32] This strategy was applied on aldehydes bearing an electron withdrawing group (nitro group and cyano group) **3.33** to afford β-hydroxy ketone **3.34**. The final α-diazo-1,3-diketone **3.35** was obtained by mild oxidation with IBX, followed by a diazo transfer reaction with *p*-ABSA. However, when using other aldehydes, the reaction becomes slow and at least one day of reaction is required to afford the corresponding β-hydroxy ketone.[33]

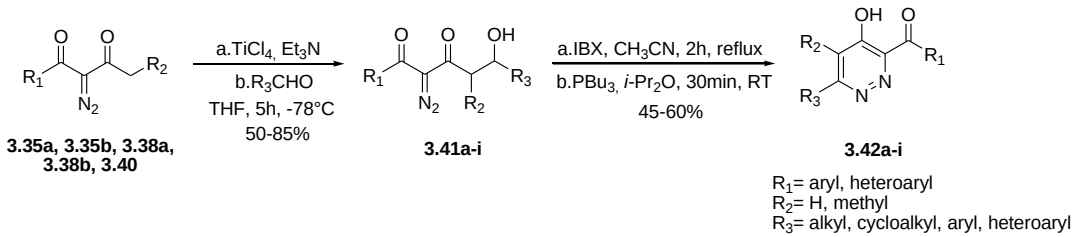
Therefore, for the other α-diazo-1,3-diketone derivatives, a strategy based on the Mukaiyama aldol reaction[34] has been used. The acetophenone derivative **3.36** was converted to the corresponding silyl enol ether at -78 °C with LiHMDS and trimethylsilyl chloride. The silyl enol ether from **3.36** reacted with acetaldehyde or propionaldehyde in the presence of titanium tetrachloride to afford **3.37**. The desired diazo compound **3.38** was obtained after oxidation with IBX and diazo transfer reaction with *p*-ABSA.



Scheme 13. Synthesis of α-diazo-1,3-diketone.

A commercially available reagent **3.39** was used to synthesize the α-diazo-1,3-diketone **3.40** by a Regitz reaction with *p*-ABSA. Five different α-diazo-1,3-diketones **3.35a**, **3.35b**, **3.38a**, **3.38b** and **3.40** became available in this way.

The α-diazo-1,3-diketone derivatives were used for the synthesis of pyridazines. The first step was the titanium aldol reaction to afford the β-hydroxy-α-diazo diketone **3.41**. Derivative **3.41** was oxidized with IBX in refluxing acetonitrile. The crude product was used without further purification in the Diaza-Wittig reaction. Treatment with tributyl phosphine in diisopropyl ether for 30 minutes led to the desired pyridazine **3.42** bearing a ketone group at position 3.

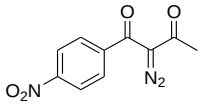
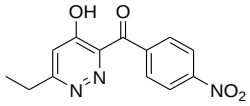
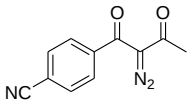
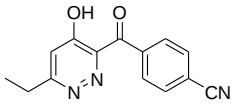
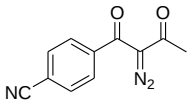
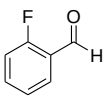
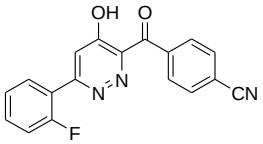
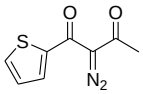
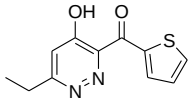
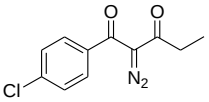
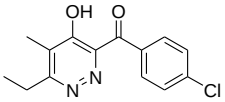
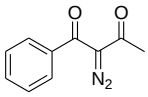
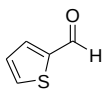
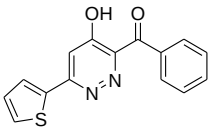
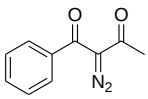
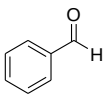
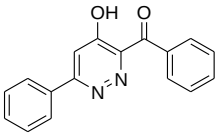
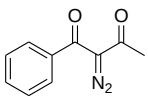
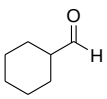
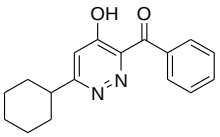
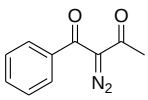
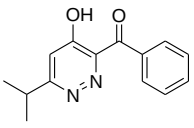


Scheme 14. Synthesis of pyridazine derivatives bearing a ketone group.

This strategy led to the synthesis of nine novel pyridazine derivatives covering different substitutions, such as several aryl ketones (Table 3, entry 1-3, 5-9) and a

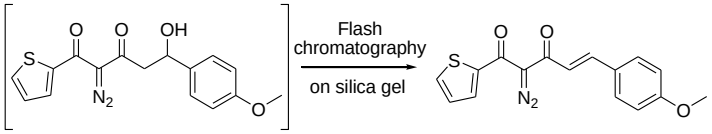
heteroaryl ketone (Table 3, entry 4). On the pyridazine ring, it was possible to introduce various R_3 groups such as alkyl (Table 3, entry 1, 2, 4, 5, 9), cycloalkyl (Table 3, entry 8), aryl (Table 3, entry 3, 7) and heteroaryl (Table 3, entry 6). The use of **3.38b** allowed the synthesis of a tetrasubstituted pyridazine (Table 3, entry 5) with R_2 being a methyl group.

Table 6. Synthesis of pyridazine derivatives bearing a ketone group at position 3.[a]

entry	α-diazo-1,3-diketone		aldehyde derivative	pyridazine		yield (%)[b]
1		**3.35a**			**3.42a**	60 [c]
2		**3.35b**			**3.42b**	57 [c]
3		**3.35b**			**3.42c**	45 [d]
4		**3.38a**			**3.42d**	55 [c]
5		**3.38b**			**3.42e**	57 [c]
6		**3.40**			**3.42f**	53 [d]
7		**3.40**			**3.42g**	48 [d]
8		**3.40**			**3.42h**	52 [d]
9		**3.40**			**3.42i**	55 [c]

[a]All reactions were performed using 1 mmol of **3.41** and 364 mg of IBX in refluxing acetonitrile for 2h, followed by a filtration to obtain the corresponding α-diazo-β-ketoester. The crude compound was stirred with 250 μL of P(*n*-Bu)$_3$ in *i*-Pr$_2$O for 30min at room temperature. [b]Isolated yield. [c]Precipitation of **3.42**. [d]Purification by flash column chromatography on silica gel.

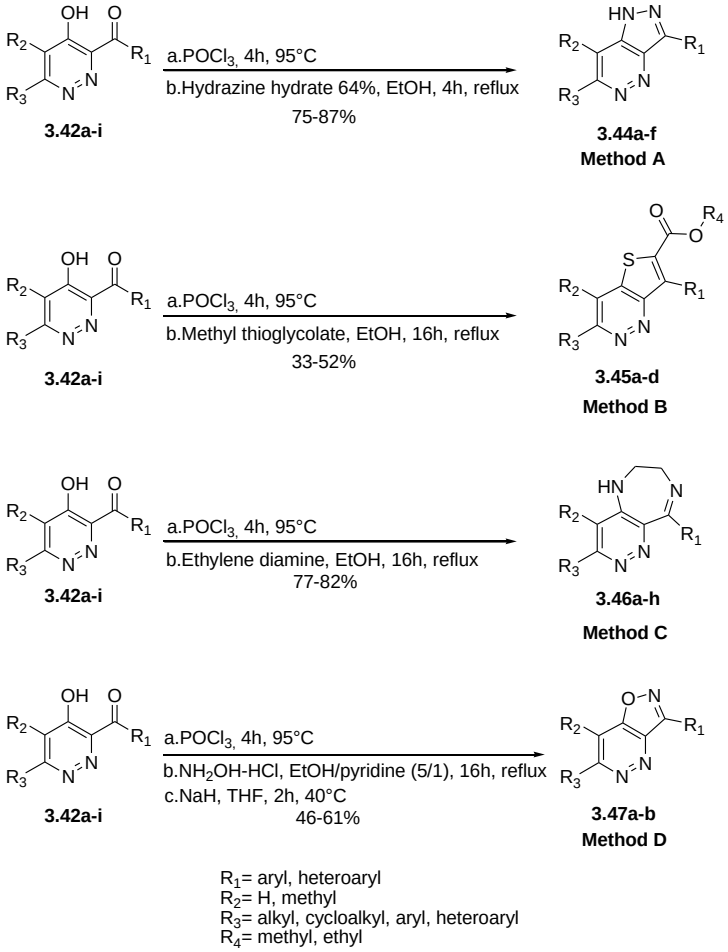
It should be noted that these aldol products **3.41** are easily degraded (water elimination) to give the corresponding enone **3.43** (complete degradation of the crude product **3.41j**, obtained from the titanium aldol reaction between **3.38a** and *p*-methoxy benzaldehyde, into the enone **3.43** during the purification by flash chromatography on silica gel).



Crude 3.41j **3.43**

Scheme 15. Degradation of aldol into enone.

The pyridazine analogues **3.42a-i** have been subjected to different cyclization methods to obtain various fused pyridazines.



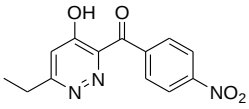
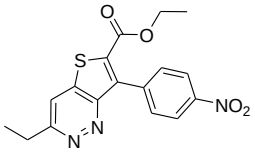
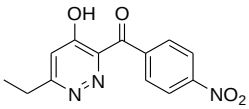
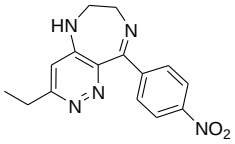
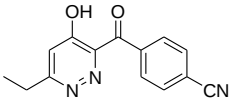
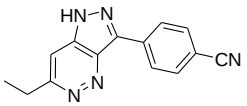
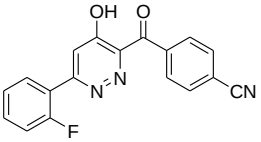
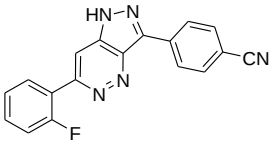
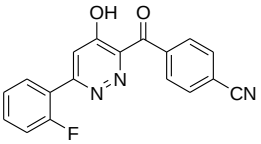
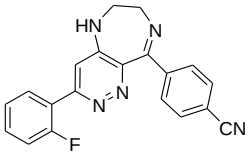
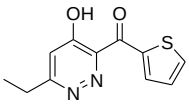
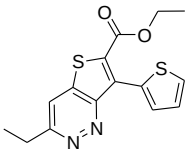
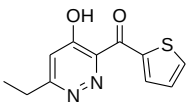
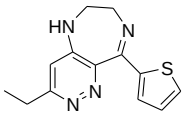
Scheme 16. Synthesis of fused pyridazines by 4 different methods (A, B, C, D).

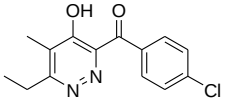
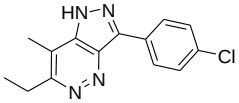
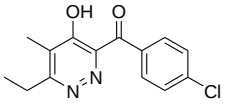
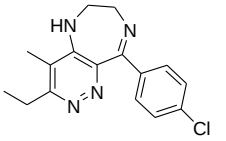
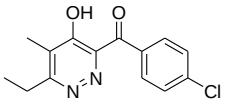
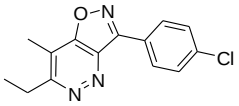
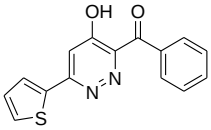
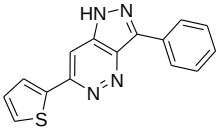
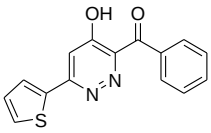
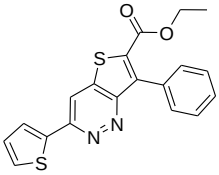
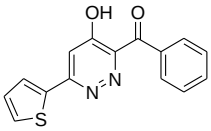
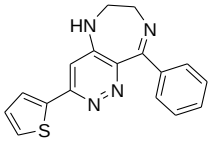
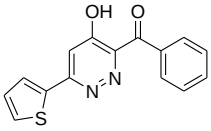
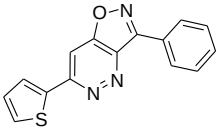
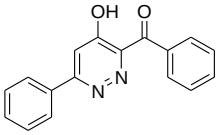
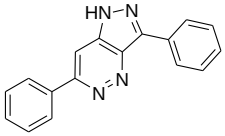
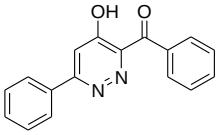
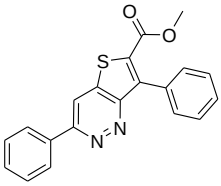
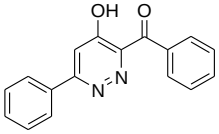
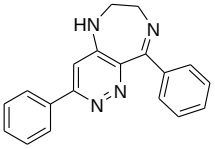
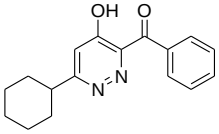
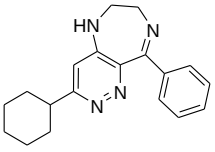
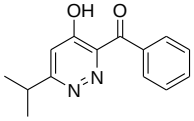
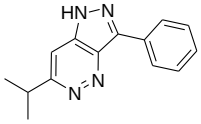
The first scaffold investigated was the pyrazolo[4,3-c]pyridazine **3.44**. After chlorination of **3.42** with phosphorous oxychloride, the crude product was refluxed with

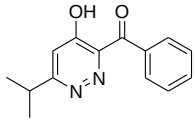
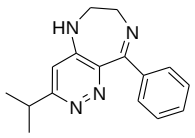
hydrazine hydrate in ethanol for 4 hours to lead to the desired product **3.44** (method A). A condensation of the methyl thioglycolate on the chlorinated pyridazine in refluxing ethanol for 16 hours led to the thieno[3,2-c]pyridazine **3.45** (method B).

In our search for novel scaffolds, we described the first synthesis of 6,7-dihydro-5H-pyridazino[4,3-e][1,4]diazepine **3.46**. Treatment of **3.42** with $POCl_3$, followed by a cyclization with ethylene diamine in refluxing ethanol for 16 hours, led to the fused pyridazine **3.46** (method C). Another novel scaffold that was synthesized with this strategy, is isoxazolo[4,5-c]pyridazine **3.47**. The ketone of the chlorinated pyridazine was converted into an oxime with hydroxylamine hydrochloride and finally cyclized by heating at 40 °C for 2 hours with sodium hydride in tetrahydrofuran to afford **3.47** (method D).

Table 7. Synthesis of fused pyridazine analogues.

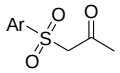
entry	pyridazine		method	fused pyridazine		yield (%)[a]
1		**3.42a**	B [b]		**3.45a**	52
2		**3.42a**	C [c]		**3.46a**	77
3		**3.42b**	A [d]		**3.44a**	83
4		**3.42c**	A [d]		**3.44b**	79
5		**3.42c**	C [c]		**3.46b**	79
6		**3.42d**	B [b]		**3.45b**	38
7		**3.42d**	C [c]		**3.46c**	81

8		**3.42e**	A [d]		**3.44c**	87
9		**3.42e**	C [c]		**3.46d**	82
10		**3.42e**	D [e]		**3.47a**	61
11		**3.42f**	A [d]		**3.44d**	81
12		**3.42f**	B [b]		**3.45c**	33
13		**3.42f**	C [c]		**3.46e**	79
14		**3.42f**	D [e]		**3.47b**	46
15		**3.42g**	A [d]		**3.44e**	79
16		**3.42g**	B [b]		**3.45d**	41
17		**3.42g**	C [d]		**3.46f**	77
18		**3.42h**	C [c]		**3.46g**	79
19		**3.42i**	A [d]		**3.44f**	75

20		**3.42i**	C [c]		**3.46h**	78

[a]Isolated yield. [b]Synthesis of thieno[3,2-c]pyridazine **3.45** with method B, see the experimental section for details. [c]Synthesis of 6,7-dihydro-5H-pyridazino[4,3-e][1,4]diazepine **3.46** with method C, see the experimental section for details. [d]Synthesis of pyrazolo[4,3-c]pyridazine **3.44** with method A, see the experimental section for details. [e]Synthesis of isoxazolo[4,5-c]pyridazine **3.47** with method D, see the experimental section for details.

During the previous paragraphs, the synthesis of new pyridazine derivatives bearing an ester group then a ketone group have been discussed, in order to cover different functional groups at the position 3 of the pyridazine, some efforts have been done to synthesize a pyridazine bearing a sulfonyl group. The first idea was the use of a sulfonyl acetone **3.47** containing an aryl substituent and the application of the strategy previously described.



sulfonyl acetone

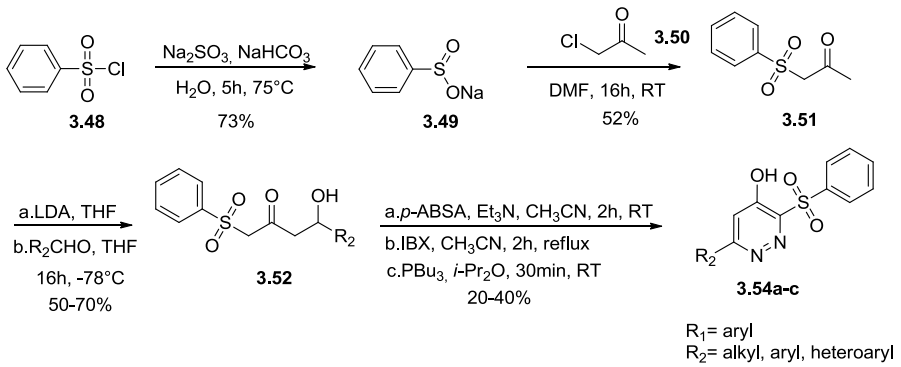
3.47

Ar= aryl group

Figure 7. Sulfonyl acetone containing an aryl group.

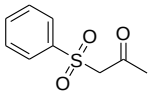
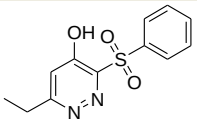
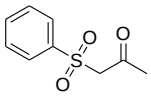
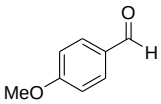
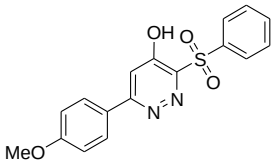
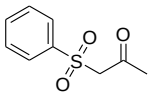
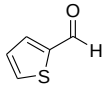
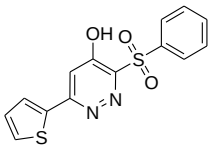
Some sulfonyl acetone derivatives are commercially available but a strategy covering the synthesis of **3.47** could be more convenient in case of particular aryl groups. The method privileged for the synthesis involved the use of sodium salt of the sulfinic acid obtained from the corresponding sulfonyl chloride.

The first step was the reduction of sulfonyl chloride **3.48** into the sulfinic acid sodium salt **3.49** with sodium sulfite and sodium hydrogenocarbonate in refluxing water.[35] The intermediate **3.49** was treated with chloro acetone **3.50** in DMF at room temperature for 16 hours to afford the sulfonyl acetone **3.51**, then a similar chemistry than the Weiler dianion previously discussed was applied to **3.51**. The dianion chemistry was used to perform an aldol reaction with an aldehyde but instead of using sodium hydride then butyl lithium to generate the dianion, two equivalents of lithium diisopropyl amide[36] were necessary before the addition of aldehyde to obtain the β-hydroxy ketone **3.52**. A diazo transfer reaction has been applied on **3.52** to get the β-hydroxy-α-diazo ketone **3.53**, followed by an oxidation with IBX in acetonitrile and the diaza Wittig reaction leading to novel sulfonyl pyridazine derivatives **3.54**.



Scheme 17. Synthesis of sulfonyl pyridazine derivatives.

Table 8. Synthesis of sulfonyl pyridazine derivatives.[a]

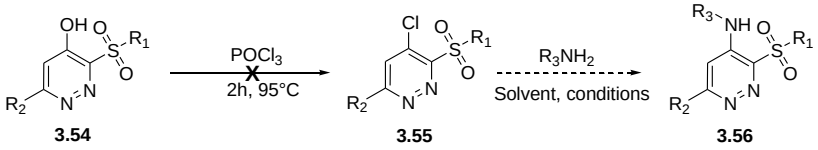
entry	sulfonyl acetone		aldehyde derivative	pyridazine		yield (%)[b]
1		**3.51**			**3.54a**	40
2		**3.51**			**3.54b**	29
3		**3.51**			**3.54c**	20

[a]All reactions were performed using 1 mmol of **3.53** and 364 mg of IBX in refluxing acetonitrile for 2h, followed by a filtration to obtain the corresponding α-diazo-β-ketoester. The crude compound was stirred with 250 μL of P(*n*-Bu)$_3$ in *i*-Pr$_2$O for 30min at room temperature. [b]Isolated yield.

The second part of the synthesis was the introduction of different groups at the position 4 of the pyridazine like the previous work by doing a chlorination of **3.54** and a nucleophilic substitution to have compound like **3.56** to start a library of sulfonyl pyridazines. Unfortunately, the chlorination attempts did not give the desired compound and instead of expected chlorinated derivative **3.55**, different side products appeared on TLC, but no separation has been done due to the complexity of the mixture.

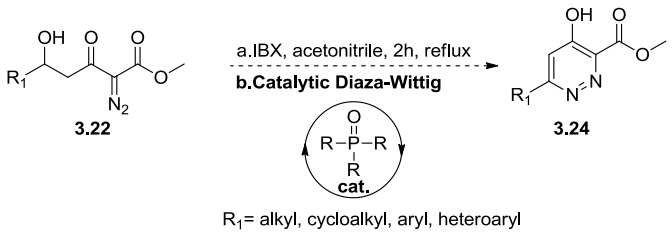
One possible explanation is that the sulfonyl group itself is a leaving group, and could have been involved during the chlorination step, moreover the position of the sulfonyl group on the pyridazine (position 3) could have played a detrimental role due the high reactivity of leaving group on this position. In fact, in pyridazine chemistry it is known that nucleophilic substitution is favorised at position 4, then position 3 and finally position 6, all these assumptions led us to think about other ways of introducing

variations on the sulfonyl pyridazine derivatives, and many efforts are currently under investigation to solve this problem.



Scheme 18. Attempt of chlorination on sulfonyl pyridazine derivatives.

The most important reaction described during this chapter was the Diaza-Wittig reaction, at the beginning the HMPT was the reagent chosen to perform the cyclization to afford the pyridazine ring. A first improvement has been done to substitute this organophosphorus specie by a less toxic reagent, after a screening of different phosphines, P(*n*-Bu)$_3$ led to the synthesis of pyridazine derivatives in shorter reaction time. By choosing the adequate solvent for the Diaza-Wittig reaction, it was possible to obtain the product as a precipitate in most of the cases.



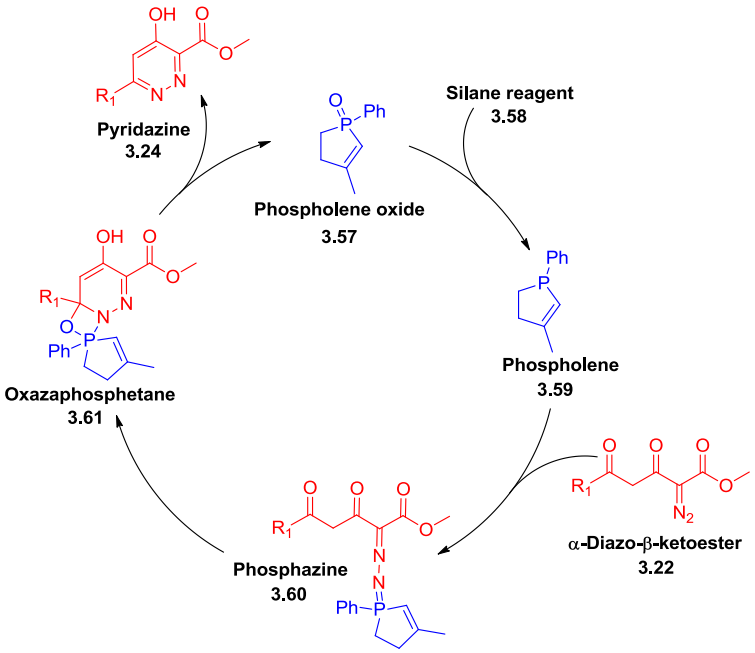
Scheme 19. Catalytic Diaza-Wittig reaction.

In our search for innovative and safe pathways, the elaboration of a catalytic Diaza-Wittig reaction became highly desirable. The first example of the synthesis of a heterocycle by using a catalytic amount of organophosphorus reagent was reported in 2008 by Marsden *et al.*.[37] An isocyanate group was needed to regenerate the phosphine oxide following a mechanism of activation, described by Campbell *et al.*,[38] for the production of carbodiimides.

Several important reactions are using organophosphorus reagents such as Appel reaction,[39] Mitsunobu reaction[40] or Wittig olefination,[41] and numerous groups have been focused on the development of a catalytic approach of these reactions. Denton *et al.* reported the first example of catalytic phosphorus mediated dichlorination of epoxides under Appel reaction conditions.[42] The group of O'Brien[43] made a breakthrough in this field by introducing a mild reduction of phosphine oxide into the corresponding phosphine by using silane as reducing reagent in contrast to previously described harsh

conditions[44] using lithium aluminium hydride, not compatible with all substrates. This reduction led to the first catalytic olefination reaction. A recent study[45] has demonstrated that the choice of the phosphine oxide is critical to achieve the reduction by silane reagent. In fact, it was shown that cyclic phosphine oxide is effectively reduced compared to acyclic phosphine oxide such as triphenyl phosphine oxide. 5-Membered phospholane gave better results and directed our work towards the use of the commercially available phospholene oxide **3.57** as catalyst.

The development of a catalytic Diaza-Wittig reaction can be divided in a four-step catalytic process. The first step is the reduction of the phospholene oxide **3.57** into the corresponding phospholene **3.59** with a silane reagent **3.58**. The second step is the formation of a phosphazine intermediate **3.60** from the reaction between **3.59** and a diazo derivative **3.22**. Then the phosphazine **3.60** is converted into an oxazaphosphetane intermediate **3.61** prior to the last step leading to the desired pyridazine **3.24** and regeneration of the phospholene oxide **3.57**.



Scheme 20. Proposed catalytic cycle for the Diaza-Wittig reaction.

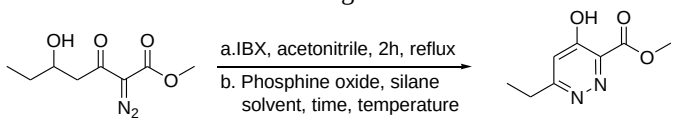
The reduction of phosphine oxide into the corresponding phosphine with silane reagent has proved to be chemoselective in the presence of aldehyde or ketone but it is the first report of this reduction in the presence of a diazo group. The reduction of the phosphine oxide did not affect the diazo group and the cyclization leading to the pyridazine could be achieved. A study on the effect of the phosphine, silane, solvent,

temperature and reaction time has been performed on the conversion of **3.22a** into **3.24a**.

In order to prove that the original reaction with stoichiometric amount of HMPT (Table 9, entry 1) cannot be catalytic, the same reaction with 25% of phosphine has been run for 48 hours, never exceeding a yield of 15% (Table 9, entry 2). A reaction with a stoichiometric amount of HMPA demonstrated that phosphine oxide alone is not enough to obtain the pyridazine (Table 9, entry 3). Likewise, using catalytic amount of triphenyl phosphine oxide or HMPA with diphenylsilane led to no formation of pyridazine **3.24a** (Table 9, entry 4-5). While varying the silane reagent and using a catalytic amount of phospholene oxide **3.57**, the use of phenylsilane led to the formation of the desired product **3.24a** in moderate yield (Table 9, entry 6). By using diphenylsilane, the pyridazine **3.24a** was obtained in excellent yields and no purification was necessary due to the precipitation of the desired pyridazine after cooling down the reaction (Table 9, entry 11-12). As a control reaction, no formation of pyridazine **3.24a** was observed in the absence of the silane reagent.

The temperature seems to be an important parameter. When the reaction was performed in DCM at room temperature, like the reaction with HMPT, **3.24a** was not formed (Table 9, entry 7). An increase of the temperature to 65 °C with acetonitrile as solvent did not give better results (Table 9, entry 8). A high temperature (100 °C) was needed to perform the first step of the catalytic process i.e. the reduction of **3.57** by the silane reagent. In DMF at 100 °C, it was possible to obtain **3.24a** in moderate yield and purification by flash chromatography on silica gel was necessary (Table 9, entry 9). Toluene is the only solvent which gives **3.24a** in excellent yields and without purification. A sealed tube was used to perform the reaction at 115 °C in toluene in order to reduce the reaction time but the product **3.24a** was obtain in lower yield (Table 9, entry 10).

Table 9. Evaluation of the phosphine, silane, solvent, temperature and reaction time on the catalytic Diaza-Wittig reaction.[a]



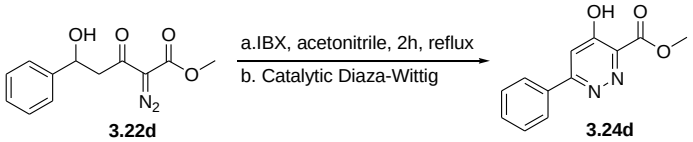
3.22a **3.24a**

entry	organophosphorus reagent	silane	solvent	temperature (°C) [b]	time (hours)	yield (%)[c]
1	HMPT[d]	-	CH_2Cl_2	RT	16	68[e]
2	HMPT[f]	-	CH_2Cl_2	RT	48	13[e]
3	HMPA[d]	-	CH_2Cl_2	RT	16	0
4	$O=PPh_3$[f]	Ph_2SiH_2	toluene	100	16	0
5	HMPA[f]	Ph_2SiH_2	toluene	100	16	0
6	**3.57**[g]	$PhSiH_3$	toluene	100	16	46[e]
7	**3.57**[g]	Ph_2SiH_2	CH_2Cl_2	RT	16	0
8	**3.57**[g]	Ph_2SiH_2	CH_3CN	65	16	0
9	**3.57**[g]	Ph_2SiH_2	DMF	100	16	71[e]
10	**3.57**[g]	Ph_2SiH_2	toluene	115[h]	16	64[i]
11	**3.57**[g]	Ph_2SiH_2	toluene	100	16	92[i]
12	**3.57**[g]	Ph_2SiH_2	toluene	100	48	95[i,j]
13	**3.57**[g]	-[k]	toluene	100	16	0

[a]See experimental section. [b]Temperature of the oil bath. [c]Yield of isolated product is quoted as an average over at least two experiments. [d]Stoichiometric amounts of organophosphorus reagent were used. [e]Purification by flash chromatography on silica gel. [f]Catalytic amounts (25 mol %) were used. [g]Catalytic amounts (10 mol %) were used. [h]Reaction was performed in a sealed tube at 115 °C. [i]Precipitation of **2a**. [j]Addition of 10 mol % of **3** to the initial amount after 24 h of reaction. [k]Control reaction without silane.

The optimum conditions for the catalytic Diaza-Wittig reaction is the use of 10 mol % of **3.57** with diphenylsilane as reducing reagent in toluene at 100 °C for 16 hours. The amount of catalyst used so far was always 10 mol % of **3.57**. To assess this novel method further, it was necessary to vary the loading of the phospholene oxide **3.57**. The conversion of **3.22d** into **3.24d** was used for this optimization process.

Table 10. Optimization of the catalytic Diaza-Wittig reaction by varying the loading of the catalyst **3.57**.[a]



3.22d **3.24d**

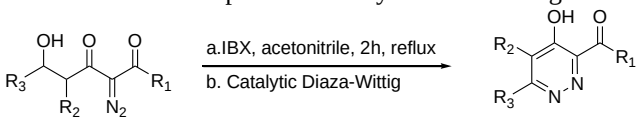
entry	loading of **3.57** (mol %)	time (hours)	yield (%)[b]
1	1	36	51
2	3	36	55
3	5	36	69
4	10	16	75
5	25	16	78

[a]See experimental section for details. [b]Yield of isolated product is quoted as an average over at least two experiments.

The catalytic Diaza-Wittig reaction was performed following the optimum conditions previously discussed and the amount of catalyst **3.57** was varied. The use of **3.22d** as starting material led to the desired product **3.24d** in 75% yield with 10 mol % of **3.57** (Table 10, entry 4). Increasing the amount of **3.57** to 25 mol % gave almost similar results (Table 10, entry 5), and a decrease below 10 mol % of the phospholene oxide **3.57** (Table 10, entry 2-3), likewise, led to product formation but in longer reaction time. The use of 1 mol % of catalyst **3.57** afforded **3.57** in 51% after 36 hours (Table 10, entry 1).

We then focused on the synthesis of a small library of pyridazine derivatives **3.24** and **3.42** to evaluate the catalytic Diaza-Wittig reaction with different substrates.

Table 11. Substrate scope of the catalytic Diaza-Wittig reaction.[a]



3.22, 3.41 **3.24, 3.42**

entry	R_1	R_2	R_3	product	yield (%)[b]
1	OMe	H	Et	**3.24a**	92[c]
2	OMe	H	Ph	**3.24d**	75[c]
3	OMe	H	Cyclohexyl	**3.24c**	79[d]
4	OMe	H	4-$MeOC_6H_4$	**3.24e**	78[c]
5	OMe	H	2-FC_6H_4	**3.24h**	82[c]
6	OMe	Me	Et	**3.24i**	81[c]
7	OMe	Me	Ph	**3.24j**	63[c]
8	OMe	Me	Cyclohexyl	**3.24k**	75[d]

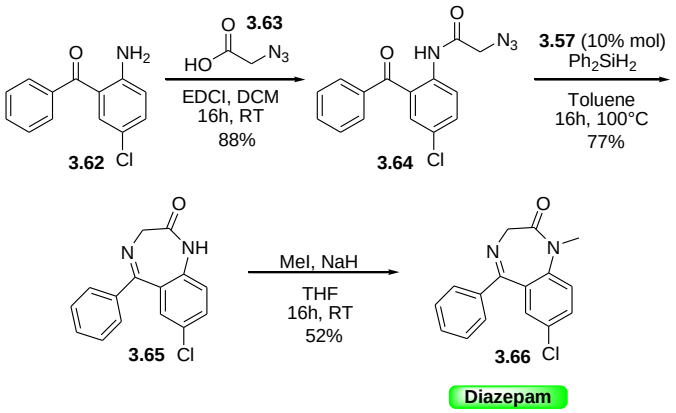
9	OMe	Me	4-MeOC_6H_4	**3.24l**	70[c]
10	OMe	Me	2-FC_6H_4	**3.24m**	78[c]
11	Ph	H	Ph	**3.42g**	53[c]
12	Ph	H	*i*-Pr	**3.42i**	74[c]

[a]See supporting information for details. [b]Yield of isolated product is quoted as an average over at least two experiments. [c]Precipitation of the pyridazine. [d]Purification by flash chromatography on silica gel.

Most of the pyridazines were obtained as precipitate in good (Table 11, entry 11) to excellent yields (Table 11, entry 1). Only compounds **3.24c** and **3.24k** needed to be purified by flash chromatography on silica gel. The catalytic Diaza-Wittig reaction was compatible with different substitutions R_3, from alkyl (**3.24a**, **3.42i**), cycloalkyl (**3.24c**, **3.24k**) to aryl (**3.24e**, **3.24m**). It was also possible to perform the reaction in the presence of different functional groups such as ester (**3.24l**) or ketone (**3.42g**). Finally, the cyclization gave access to trisubstituted (**3.24h**) and tetrasubstituted (**3.24i**) pyridazines by varying R_2.

In order to extend this novel catalytic method to other substrates, our work was directed to the synthesis of 1,4-benzodiazepine.[46] The Diaza-Wittig reaction is based on the reaction between a diazo derivative and a carbonyl group to generate substituted hydrazone. The Aza-Wittig[47] reaction leads to substituted imines from azido derivatives and carbonyl groups. In both cases the key reagent is the organophosphorus specie. From this observation, we decided to apply similar conditions as previously discussed, to perform a novel catalytic Aza-Wittig reaction to synthesize 1,4-benzodiazepine and especially the Diazepam[48] used to treat anxiety or insomnia.

The synthesis started with the EDCI amide coupling of 2-amino-5-chloro benzophenone **3.62** and azido acetic acid **3.63** at room temperature to lead to the azido acetamido derivative **3.64** in 88% yield. The next step is the intramolecular catalytic Aza-Wittig between the azide and the ketone. The 1,4-benzodiazepine **3.65** was obtained after purification by flash chromatography on silica gel in 77% yield. The final methylation of **3.65** with iodomethane afforded the Diazepam **3.66**.



Scheme 21. Synthesis of Diazepam by a catalytic Aza-Wittig reaction.

3. Biological evaluation

The different libraries of pyridazine derivatives and related fused heterocycles developed in this chapter have been screened towards selected kinases of ongoing projects of Galapagos. The compounds were first tested in an enzymatic assay at constant concentration and for the analogues displaying an interesting percentage of inhibition for a specified kinase, a further assay was performed at different concentrations in order to obtain the half maximal inhibitory concentration IC_{50}.

Table 12. Biological evaluation of pyridazine derivatives (N/A not active).

entry	compounds	CDK9 (% inhibition at 10 μM)	IC_{50} (nM)	TGF-β II (% inhibition at 10 μM)	IC_{50} (nM)
1	**3.29a**	5	N/A	16	N/A
2	**3.29b**	N/A	N/A	4	N/A
3	**3.29c**	27	N/A	10	N/A
4	**3.29d**	94	290	9	N/A
5	**3.29e**	9	N/A	13	N/A
6	**3.29f**	53	N/A	16	N/A
7	**3.29g**	14	N/A	21	N/A
8	**3.29h**	N/A	N/A	N/A	N/A
9	**3.29i**	N/A	N/A	N/A	N/A
10	**3.29j**	15	N/A	9	N/A
11	**3.29k**	N/A	N/A	N/A	N/A
12	**3.29l**	N/A	N/A	5	N/A

13	**3.29m**	11	N/A	23	N/A
14	**3.29n**	N/A	N/A	N/A	N/A
15	**3.29o**	23	N/A	8	N/A
16	**3.29p**	4	N/A	16	N/A
17	**3.29q**	N/A	N/A	4	N/A
18	**3.29r**	N/A	N/A	N/A	N/A
19	**3.29s**	N/A	N/A	21	N/A
20	**3.31a**	52	N/A	5	N/A
21	**3.31b**	16	N/A	N/A	N/A

entry	compounds	GSK3 (% inhibition at 20 μM)	IC_{50} (nM)	IRAK4 (% inhibition at 20 μM)	IC_{50} (nM)
22	**3.44a**	78	>4000	55	>20000
23	**3.44b**	92	783	76	>20000
24	**3.44c**	87	>4000	95	3932
25	**3.44d**	75	>4000	94	2150
26	**3.44e**	83	>4000	97	1829
27	**3.44f**	17	>20000	9	>20000
28	**3.45a**	28	>20000	7	>20000
29	**3.45b**	N/A	N/A	N/A	N/A
30	**3.45c**	40	>20000	7	>20000
31	**3.45d**	16	>20000	10	>20000
32	**3.46a**	12	>20000	7	>20000
33	**3.46b**	13	>20000	12	>20000
34	**3.46c**	N/A	N/A	N/A	N/A
35	**3.46d**	20	>20000	4	>20000
36	**3.46e**	6	>20000	13	>20000
37	**3.46f**	16	>20000	10	>20000
38	**3.46g**	22	>20000	13	>20000
39	**3.46h**	15	>20000	30	>20000
40	**3.47a**	12	>20000	8	>20000
41	**3.47b**	21	>20000	8	>20000

42	**3.54a**	30	>20000	13	>20000
43	**3.54b**	39	>20000	10	>20000
44	**3.54c**	22	>20000	9	>20000

The first screening concerned the derivatives of pyridazine containing a substituted amide group **3.29**. These analogues have been assayed towards CDK9 and TGF-β II receptor and only the compound **3.29d** displayed an interesting half maximal inhibitory concentration IC_{50} = 290 nM. Most of the other pyridazines did not lead to a percentage of inhibition higher than 50 % and thus have not been screened at different concentrations but it can be observed from **3.29d** that an aliphatic amine substitutiuon such as ethylamine at the position 4 gave better response compared to cyccloalkyl or aryl amines. Moreover, the replacement of a N-linker at the position 4 of the pyridazine by an aryl directly connected without linker **3.31** did not improve the characteristic of the analogues. A series of fused pyridazine has been towards two different kinases the GSK3 and IRAK4, amongst them only the derivatives based on the pyrazolo[4,3-c]pyridazine scaffold **3.44** have led to more than 70 % of inhibition at 20 μM. Of particular interest, the compound **3.44b** gave an IC_{50} value of 783 nM against GSK3 and the compound **3.44e** with an IC_{50} of 1829 nM towards IRAK4. The other libraries of fused rings **3.45**, **3.46**, **3.47** did not display significant biological activity. The three examples of sulfonyl pyridazine derivatives **3.54** did not give inhibition above 50 % for the two targeted receptors.

4. Conclusion

In conclusion, we have first developed a concise and convenient procedure for the synthesis of 6-substituted-4-hydroxy-3-methoxycarbonyl pyridazines **3.24** involving a Diaza-Wittig reaction as key-step, allowing the introduction of a variety of substitutions from alkyl, cycloalkyl to aryl and heteroaryl at the C6 position. This method, likewise, allows the synthesis of pyridazines with different substitutions at C4 and C6 positions, and a library has been synthesized varying three different points of substitutions to obtain pyridazine analogues **3.29** and **3.31**.

Then, the method has been improved by using the Calter method for the formation of the aldol intermediate **3.22** and **3.41**, the second improvement was the replacement of

the toxic HMPT by P(*n*-Bu)$_3$ to perform the Diaza-Wittig reaction in a shorter reaction time and with better yields.

In a second part, based on the methodology developed and starting from 1,3-diketones instead of methyl acetoacetate, it was possible to synthesize novel pyridazines bearing a ketone group **3.42** with different substituents at position 6. These pyridazine analogues were used for the development of a 20-member library of novel biheterocyclic compounds such as pyrazolo[4,3-c]pyridazine **3.44** and thieno[3,2-c]pyridazine **3.45**. It is also the first report dealing with the synthesis of 6,7-dihydro-5H-pyridazino[4,3-e][1,4]diazepine **3.46** and isoxazolo[4,5-c]pyridazine **3.47**.

The synthesis of pyridazine bearing a sulfonyl group at the position 3 has also been elaborated following the same strategy and using sulfonyl acetone **3.51** as starting material but the synthesis of library was not possible due to the non-chlorination of the hydroxyl group of the pyridazine derivative **3.54**.

Finally, we reported the first catalytic Diaza-Wittig reaction and its application for the synthesis of pyridazine analogues **3.24** and **3.42**. The reaction tolerated various substituents and functional groups and delivered most of the derivatives without purification. This methodology also led to a novel catalytic Aza-Wittig reaction and was applied to the synthesis of the 1,4-benzodiazepine Diazepam **3.66**. These two methods represent new catalytic approaches to relevant heterocycles known as "privileged structures" for the pharmaceutical industry.

5. Experimental section

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 ºC) under a nitrogen or argon atmosphere. Reaction temperatures are reported as bath temperature. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Compounds were visualized with UV light (λ = 254 nm). Products were purified by flash chromatography on ICN silica gel 63-200, 60 Å. Melting points were obtained on a melting point apparatus Electrothermal IA9200 with open capillary tubes. ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz, 500 MHz and 600 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as the solvent. The ^{1}H and ^{13}C chemical shifts were referenced to residual solvent signals at δ H/C 7.26/77.00 ($CDCl_3$), 3.31/49.10 and 2.50/39.50 (DMSO-d_6) relative to TMS as internal standard. Coupling constants *J* [Hz] were directly taken from the spectra. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High resolution mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were

infused at 3 μL/min and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Electrospray MS spectra were obtained on a Micromass platform LC/MS spectrometer. Column used for all LC/MS analysis: Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm ID x 50 mm L. All the methods are using MeCN/H_2O gradients. Water contains either 0.1 % TFA or 0.1 % NH_3.

Diethyl 2-diazo-3-oxopentanedioate (3.12)

To a solution of compound **3.11** (1 g, 4.95 mmol) in 20 mL of acetonitrile under argon at 0 °C was added successively triethylamine (895 μL, 6.44 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (1.2 g, 4.95 mmol). The reaction mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 100 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the desired compound **3.12** in 85% yield as yellow oil. Data for **3.12**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.24 (q, *J* = 7.4 Hz, 2H), 4.16 (q, *J* = 7.3 Hz, 2H), 3.78 (s, 2H), 1.32 (t, *J* = 7.3 Hz, 3H), 1.24 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 183.2, 164.3, 159.0, 62.1, 61.7, 60.9, 45.2, 15.1, 14.8.

Ethyl 4,6-dihydroxypyridazine-3-carboxylate (3.14)

To a solution of **3.12** (1 g, 4.38 mmol) in 15 mL of Et_2O under argon was added PPh_3 (1.7 g, 6.44 mmol) at room temperature. The reaction mixture was stirred for 24 hours and concentrated in vacuo to an orange oil, which was dissolved in AcOH/H_2O (4:1) and refluxed for further 24 hours. The reaction was allowed to cool to room temperature and the solvent were evaporated to obtain a yellow oil purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 9:1) to afford the desired compound **3.14** in 30% yield as pale yellow solid. Data for **3.14**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.02 (br. s, 1H), 11.59 (br. s, 1H), 6.02 (s, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.5, 162.6, 158.5, 135.8, 105.7, 61.8, 14.1.

Ethyl 4,6-dichloropyridazine-3-carboxylate (3.15)

3.14 (184 mg, 1 mmol) was dissolved in 5 mL of phosphorus oxychloride at room temperature. The reaction mixture was refluxed for 4 hours after which the color of the solution became brown. The flask was allowed to cool to room temperature and the excess of phosphorus oxychloride was concentrated in vacuo to a brown oil. The oil was dissolved in 15 mL of EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated to afford crude **3.15** in 75% yield as brown oil used directly without further purification. Data for **3.15**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.58 (s, 1H), 4.47 (q, *J* = 7.1 Hz, 2H), 1.36 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7, 159.3, 147.2, 141.0, 109.8, 62.3, 14.1.

General procedure for preparation of 5-hydroxy-5-substituted-3-oxo-pentanoates 3.20:

To a suspension of 60% sodium hydride (48 mg, 1.2 mmol) in 60 mL of dry THF under argon at 0°C was slowly added methyl acetoacetate **3.19** (108 μL, 1.0 mmol). After hydrogen evolution had ceased (30 minutes), the reaction mixture was cooled to -78°C and 2.5 M butyllithium (480 μL, 1.2 mmol). The mixture was stirred for 30 minutes, after which time aldehyde was added and the mixture stirred for 5 hours. The reaction was allowed to warm to room temperature and was diluted with 100 mL of saturated aqueous NH_4Cl and extracted 2 times with 100 mL of ethyl acetate. The organic layer was dried over Na_2SO_4, concentrated in vacuo, and purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the desired compound **3.20**.

Methyl 5-hydroxy-3-oxoheptanoate (3.20a)

Yellow oil, 60% yield; Data for **3.20a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.98-4.07 (m, 1H), 3.75 (s, 3H), 3.50 (s, 2H), 2.62-2.76 (m, 2H), 1.48-1.55 (m, 2H), 0.94-0.97 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.6, 167.3, 68.9, 52.4, 49.7, 49.2, 29.3, 11.7. HRMS calcd for $C_8H_{14}O_4$ $[M+Na]^+$ 197.0785, found 197.0791.

Methyl 5-hydroxy-6-methyl-3-oxoheptanoate (3.20b)

Yellow oil, 51% yield; Data for **3.20b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85-3.87 (m, 1H), 3.75 (s, 3H), 3.53 (s, 2H), 2.73 (dd, *J* = 17.2, 2.6 Hz, 1H), 2.65 (dd, *J* = 17.3, 9.4 Hz, 1H), 1.69-1.72 (m, 1H), 0.92-0.95 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.0, 167.4, 72.1, 52.4, 49.7, 46.7, 33.0, 18.3, 17.6. HRMS calcd for $C_9H_{16}O_4$ $[M+Na]^+$ 211.0941, found 211.0940.

Methyl 5-cyclohexyl-5-hydroxy-3-oxopentanoate (3.20c)

Yellow oil, 37% yield; Data for **3.20c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (m, 1H), 3.75 (s, 3H), 3.52 (s, 2H), 2.73 (dd, *J* = 17.3, 2.6 Hz, 1H), 2.67 (dd, *J* = 17.4, 9.6 Hz, 1H), 1.82-1.85 (m, 1H), 1.75-1.77 (m, 3H), 1.64-1.68 (m, 2H), 1.33-1.39 (m, 1H), 1.11-1.27 (m, 4H), 1.07-0.97 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.1, 167.4, 71.6, 52.4, 49.7, 46.8, 42.9, 28.8, 28.1, 26.3, 26.1, 25.9. HRMS calcd for $C_{12}H_{20}O_4$ $[M+Na]^+$ 251.1254, found 251.1249.

Methyl 5-hydroxy-3-oxo-5-phenylpentanoate (3.20d)

Yellow oil, 55% yield; Data for **3.20d**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.36 (m, 4H), 7.28-7.30 (m, 1H), 5.19 (dd, *J* = 9.4, 2.6 Hz, 1H), 3.74 (s, 3H), 3.51 (s, 2H), 3.00 (dd, *J* = 17.5, 9.4 Hz, 1H), 2.91 (dd, *J* = 17.3, 3.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.8, 167.3, 142.4, 128.6, 127.8, 125.6, 69.8, 52.5, 51.5, 49.6. HRMS calcd for $C_{12}H_{14}O_4$ $[M+Na]^+$ 245.0785, found 245.0778.

Methyl 5-hydroxy-5-(4-methoxyphenyl)-3-oxopentanoate (3.20e)

Yellow oil, 40% yield; Data for **3.20e**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.29 (m, 2H), 6.87-6.89 (m, 2H), 5.15 (dd, *J* = 9.4, 3.2 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.50 (s, 2H), 3.00 (dd, *J* = 17.2, 9.3 Hz,

1H), 2.88 (dd, *J* = 17.2, 3.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.7, 167.3, 159.2, 134.6, 126.9, 114.0, 69.5, 55.3, 52.4, 51.5, 49.7. HRMS calcd for $C_{13}H_{16}O_5$ $[M+Na]^+$ 275.0890, found 275.0885.

Methyl 5-(furan-2-yl)-5-hydroxy-3-oxopentanoate (3.20f)

Yellow oil, 25% yield; Data for **3.20f**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.38 (m, 1H), 6.33 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.28 (d, *J* = 3.3 Hz, 1H), 5.20 (dd, *J* = 8.9, 3.3 Hz, 1H), 3.75 (s, 3H), 3.54 (s, 2H), 3.18 (dd, *J* = 17.6, 8.9 Hz, 1H), 3.04 (dd, *J* = 17.6, 3.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.1, 167.2, 154.4, 142.2, 110.3, 106.5, 63.5, 52.5, 49.5, 47.7. HRMS calcd for $C_{10}H_{12}O_5$ $[M+Na]^+$ 235.0577, found 235.0576.

Methyl 5-hydroxy-3-oxo-5-(thiophen-2-yl)-pentanoate (3.20g)

Yellow oil, 30% yield; Data for **3.20g**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.27 (m, 1H), 6.96-6.98 (m, 2H), 5.44 (dd, *J* = 8.9, 3.1 Hz, 1H), 3.75 (s, 3H), 3.53 (s, 2H), 3.13 (dd, *J* = 17.6, 8.9 Hz, 1H), 3.05 (dd, *J* = 17.5, 3.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.2, 167.1, 146.1, 126.7, 124.9, 123.7, 66.0, 52.5, 51.3, 49.6. HRMS calcd for $C_{10}H_{12}O_4S_1$ $[M+Na]^+$ 251.0349, found 251.0344.

General procedure for preparation of 3.22 by diazo transfer :

To a solution of compound **3.20** (1 mmol) in 30 mL of acetonitrile under argon at 0°C was added successively triethylamine (181 μL, 1.3 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (240 mg, 1 mmol). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 50 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the desired compound **3.22**.

Methyl 2-diazo-5-hydroxy-3-oxoheptanoate (3.22a)

Yellow oil, 90% yield; Data for **3.22a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.01-4.02 (m, 1H), 3.85 (s, 3H), 3.10 (dd, *J* = 17.1, 2.4 Hz, 1H), 3.04 (d, J = 3.3 Hz, 1H), 2.91 (dd, *J* = 17.1, 9.4 Hz, 1H), 1.50-1.61 (m, 2H), 0.98 (t, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.0, 161.7, 69.4, 52.3, 46.3, 29.5, 9.8. HRMS calcd for $C_8H_{12}N_2O_4$ $[M+Na]^+$ 223.0690, found 223.0690.

Methyl 2-diazo-5-hydroxy-6-methyl-3-oxoheptanoate (3.22b)

Yellow oil, 90% yield; Data for **3.22b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.80-3.85 (m, 4H), 3.08 (dd, *J* = 17.0, 2.3 Hz, 1H), 2.90-2.95 (m, 2H), 1.72-1.78 (m, 1H), 0.95-0.98 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.4, 161.7, 72.8, 52.3, 43.9, 33.3, 18.4, 17.7. HRMS calcd for $C_9H_{14}N_2O_4$ $[M+Na]^+$ 237.0846, found 237.0847.

Methyl 2-diazo-5-cyclohexyl-5-hydroxy-3-oxopentanoate (3.22c)

Yellow oil, 87% yield; Data for **3.22c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (m, 4H), 3.09 (dd, *J* = 16.8, 2.3 Hz, 1H), 2.94 (dd, *J* = 16.9, 9.9 Hz, 2H), 1.87-1.89 (m, 1H), 1.75-1.78 (m, 2H), 1.65-1.71 (m, 2H), 1.38-1.44 (m, 1H), 1.00-1.28 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.4, 161.7, 72.1, 52.3, 44.0, 43.2, 28.7, 28.2, 26.4, 26.1, 26.0. HRMS calcd for $C_{12}H_{18}N_2O_4$ $[M+Na]^+$ 277.1159, found 277.1159.

Methyl 2-diazo-5-hydroxy-3-oxo-5-phenylpentanoate (3.22d)

Yellow oil, 90% yield; Data for **3.22d**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.41 (m, 2H), 7.34-7.37 (m, 2H), 7.26-7.29 (m, 1H), 5.20-5.22 (m, 1H), 3.83 (s, 3H), 3.39 (d, *J* = 3.1 Hz, 1H), 3.27-3.29 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.1, 161.5, 142.6, 128.5, 127.6, 125.7, 70.2, 52.4, 48.6. HRMS calcd for $C_{12}H_{12}N_2O_4$ $[M+Na]^+$ 271.0690, found 271.0687.

Methyl 2-diazo-5-hydroxy-5-(4-methoxyphenyl)-3-oxopentanoate (3.22e)

Yellow oil, 80% yield; Data for **3.22e**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.33 (m, 2H), 6.87-6.89 (m, 2H), 5.13-5.17 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.22-3.32 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.1, 161.6, 159.1, 134.9, 127.0, 113.9, 69.9, 55.3, 52.3, 48.5. HRMS calcd for $C_{13}H_{14}N_2O_5$ $[M+Na]^+$ 301.0795, found 301.0795.

Methyl 2-diazo-5-(furan-2-yl)-5-hydroxy-3-oxopentanoate (3.22f)

Yellow oil, 62% yield; Data for **3.22f**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.38 (m, 1H), 6.33 (dd, *J* = 3.4, 1.8 Hz, 1H), 6.30 (d, *J* = 3.5 Hz, 1H), 5.23 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.85 (s, 3H), 3.50 (dd, *J* = 17.4, 9.0 Hz, 1H), 3.32 (dd, *J* = 17.4, 3.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.5, 161.5, 154.8, 142.1, 110.2, 106.2, 64.0, 52.4, 45.0. HRMS calcd for $C_{10}H_1N_2O_5$ $[M+Na]^+$ 261.0482, found 261.0485.

Methyl 2-diazo-5-hydroxy-3-oxo-5-(thiophen-2-yl)-pentanoate (3.22g)

Yellow oil, 85% yield; Data for **3.22g**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.27 (m, 1H), 6.99-7.00 (m, 1H), 6.96-6.98 (m, 1H), 5.45-5.47 (m, 1H), 3.85 (s, 3H), 3.57 (d, J = 4.1 Hz, 1H), 3.45 (dd, *J* = 17.3, 8.9 Hz, 1H), 3.40 (dd, *J* = 17.3, 3.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.7, 161.5, 146.4, 126.7, 124.7, 123.5, 66.5, 52.4, 48.2. HRMS calcd for $C_{10}H_{10}N_2O_4S_1$ $[M+Na]^+$ 277.0254, found: 277.0257.

General procedure for the preparation of pyridazine derivatives 3.24 with HMPT:

To a solution of **3.22** (1 mmol) in 10 mL of acetonitrile under argon was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was allowed to warm to room temperature and then filtered. The filtrate was concentrated to afford the α-diazo-β-ketoester **3.23** used directly without further purification for the next step.

To a solution of α-diazo-β-ketoester in 10 mL of CH_2Cl_2 was added HMPT (hexamethylphosphorous triamide) (183 μL, 1 mmol). The reaction mixture was stirred under argon at room temperature for 16 hours. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with the addition of water. The organic layer was washed with water (2 x 10 mL), dried over Na_2SO_4 and concentrated in vacuo. A purification by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) afforded the desired pyridazine **3.24**.

Methyl 6-ethyl-4-hydroxypyridazine-3-carboxylate (3.24a)

Pale yellow solid, 68% yield; Data for **3.24a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.37 (br. s, 1H), 6.40 (s, 1H), 3.80 (s, 3H), 2.56 (m, 2H), 1.19 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.8, 164.2, 156.6, 148.6, 116.2, 52.4, 24.0, 12.5.

Methyl 4-hydroxy-6-isopropylpyridazine-3-carboxylate (3.24b)

Yellow solid, 75% yield; Data for **3.24b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.38 (br. s, 1H), 6.42 (s, 1H), 3.81 (s, 3H), 2.85 (m, 1H), 1.22 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.9, 164.2, 160.4, 148.6, 114.4, 52.4, 30.2, 21.0.

Methyl 6-cyclohexyl-4-hydroxypyridazine-3-carboxylate (3.24c)

Yellow solid, 62% yield; Data for **3.24c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.37 (s, 1H), 3.80 (s, 3H), 1.77-1.84 (m, 4H), 1.67-1.69 (m, 1H), 1.18-1.46 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.9, 164.1, 159.3, 148.5, 114.9, 52.4, 30.9, 25.7, 25.1.

Methyl 4-hydroxy-6-phenylpyridazine-3-carboxylate (3.24d)

Brown solid, 60% yield; Data for **3.24d**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.72 (br. s, 1H), 7.79-7.81 (m, 2H), 7.60 (m, 3H), 6.86 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.1, 152.2, 148.4, 131.2, 131.0, 129.2, 127.4, 115.9, 52.4.

Methyl 4-hydroxy-6-(4-methoxyphenyl)pyridazine-3-carboxylate (3.24e)

Orange solid, 45% yield; Data for **3.24e**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 6.81 (s, 1H), 3.84 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.2, 161.7, 148.2, 129.0, 122.8, 115.0, 114.7, 55.6, 52.5.

Methyl 6-(furan-2-yl)-4-hydroxypyridazine-3-carboxylate (3.24f)

Brown solid, 27% yield; Data for **3.24f**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04 (m, 1H), 7.44-7.45 (m, 1H), 6.87 (s, 1H), 6.78 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.9, 146.8, 113.3, 113.1, 111.5, 52.4.

Methyl 4-hydroxy-6-(thiophen-2-yl)-pyridazine-3-carboxylate (3.24g)

Brown solid, 33% yield; Data for **3.24g**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H), 7.76-7.77 (m, 1H), 7.58-7.59 (m, 1H), 7.32 (s, 1H), 7.19 (dd, *J* = 5.0, 3.8 Hz, 1H), 4.13 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.1, 160.0, 157.7, 139.6, 136.6, 131.0, 128.4, 127.9, 108.2, 53.5.

Methyl 2-diazo-3-oxobutanoate (3.25)

To a solution of compound **3.19** (5 g, 43.06 mmol) in 30 mL of acetonitrile under argon at 0 °C was added successively triethylamine (7.8 mL, 55.98 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (10.4 g, 43.06 mmol). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 100 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the desired compound **3.25** in 82% yield as yellow oil. Data for **3.25**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.65 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.3, 161.5, 52.3, 27.9.

General procedure for the preparation of 3.22 by titanium aldol reaction:

To a solution of **3.25** (1 g, 7.04 mmol) in 50 mL of CH_2Cl_2 under argon at -78 °C was added dropwise $TiCl_4$ (849 µL, 7.74 mmol) followed by Et_3N (1.08 mL, 7.74 mmol). The resulting red solution was stirred at -78 °C for 1 hour, after which a solution of aldehyde (6.34 mmol) in CH_2Cl_2 was slowly added. The reaction mixture was stirred at -78 °C for 4 hours and then the reaction was quenched with 50 mL of saturated aqueous NH_4Cl and warmed to room temperature. The organic layer was separated and then washed with 40 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted with 50 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the aldol product **3.22**.

General procedure for the preparation of pyridazine derivatives 3.24 with P(*n*-Bu)$_3$:

To a solution of **3.22** (1 mmol) in 10 mL of acetonitrile under argon was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was allowed to warm to room temperature and then filtered. The filtrate was concentrated to afford the α-diazo-β-ketoester **3.23** used directly without further purification for the next step.

To a solution of α-diazo-β-ketoester in 20 mL of *i*-Pr_2O was added P(*n*-Bu)$_3$ (250 µL, 1 mmol). The reaction mixture was stirred under argon at room temperature for 30 minutes, which after this time a precipitate was formed. The suspension was filtered, washed with *i*-Pr_2O and dried to afford **3.24**.

General procedure for the preparation of pyridazine derivatives 3.27:

3.24 (1.0 mmol) was dissolved in 5 mL of phosphorus oxychloride at room temperature. The reaction mixture was heated at 95 °C for 4 hours after which the color of the solution became brown. The flask

was allowed to cool to room temperature and the excess of phosphorus oxychloride was concentrated in vacuo to a brown oil. The oil was dissolved in 15 mL of EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated to afford crude **3.26** as brown oil used directly without further purification.

To a solution of **3.26** in 5 mL of dioxane under argon was added an amine (1.1 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (5-48 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 8:2) to afford the amino substituted pyridazine **3.27**.

Methyl 4-(ethylamino)-6-phenylpyridazine-3-carboxylate (3.27a)

Beige solid, 85% yield; Data for **3.27a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03-8.06 (m, 2H), 7.87 (br. s, NH), 7.46-7.48 (m, 3H), 6.94 (s, 1H), 4.00 (s, 3H), 3.25-3.32 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 160.8, 158.2, 147.0, 134.5, 129.6, 128.2, 114.0, 101.9, 52.2, 35.7, 14.2.

Methyl 4-(ethylamino)-6-(4-methoxyphenyl)pyridazine-3-carboxylate (3.27b)

Beige solid, 83% yield; Data for **3.27b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16 (d, *J* = 8.9 Hz, 2H), 7.79 (t, *J* = 5.4 Hz, NH), 7.26 (s, 1H), 7.09 (d, *J* = 9.0 Hz, 2H), 3.92 (s, 3H), 3.84 (s, 3H), 3.39-3.45 (m, 2H), 1.23 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.1, 161.1, 157.6, 146.8, 134.2, 128.9, 128.5, 114.3, 102.2, 55.4, 52.3, 36.0, 13.8.

Methyl 4-(cyclohexylamino)-6-(furan-2-yl)pyridazine-3-carboxylate (3.27d)

Orange solid, 75% yield; Data for **3.27d**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.97-7.98 (m, 1H), 7.94 (d, *J* = 8.0 Hz, NH), 7.38 (d, *J* = 3.4 Hz, 1H), 7.14 (s, 1H), 6.74-6.75 (m, 1H), 3.92 (s, 3H), 3.68-3.72 (m, 1H), 1.91-1.94 (m, 2H), 1.67-1.70 (m, 2H), 1.57-1.61 (m, 1H), 1.42-1.49 (m, 2H), 1.26-1.37 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.3, 150.6, 150.5, 145.8, 145.7, 133.8, 112.7, 111.8, 100.7, 52.5, 48.9, 31.6, 25.1, 23.9.

Methyl 6-phenyl-4-(phenylamino)pyridazine-3-carboxylate (3.27f)

Brown solid, 52% yield; Data for **3.27f**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (br. s, NH), 7.96-7.98 (m, 2H), 7.50-7.53 (m, 3H), 7.47-7.49 (m, 2H), 7.43-7.44 (m, 3H), 7.28-7.31 (m, 1H), 3.99 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.7, 158.5, 144.7, 137.5, 136.2, 136.0, 130.3, 129.9, 129.0, 127.2, 125.9, 123.9, 104.9, 52.7.

Methyl 6-cyclohexyl-4-(phenylamino)pyridazine-3-carboxylate (3.27g)

Yellow solid, 57% yield; Data for **3.27g**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (br. s, NH), 7.46 (t, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 7.4 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 6.93 (s, 1H), 3.94 (s, 3H), 2.71-2.77 (m, 1H), 1.75-1.83 (m, 4H), 1.66-1.69 (m, 1H), 1.17-1.47 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.9, 166.8, 144.5, 137.6, 135.9, 129.8, 125.7, 123.8, 105.4, 52.6, 43.9, 31.9, 25.9, 25.5.

Methyl 4-(phenylamino)-6-(thiophen-2-yl)pyridazine-3-carboxylate (3.27h)

Orange solid, 50% yield; Data for **3.27h**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.52 (br. s, NH), 7.78-7.79 (m, 1H), 7.72-7.73 (m, 1H), 7.48 (t, *J* = 7.4 Hz, 2H), 7.40-7.44 (m, 3H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.17-7.19 (m, 1H), 3.97 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.5, 154.2, 144.6, 139.8, 137.4, 136.0, 130.8, 129.9, 128.6, 127.9, 125.9, 123.8, 102.5, 52.7.

Methyl 6-ethyl-4-(3,4,5-trimethoxyphenylamino)-pyridazine-3-carboxylate (3.27i)

Orange solid, 62% yield; Data for **3.27i**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (br. s, NH), 7.36 (s, 2H), 7.28 (s, 1H), 3.95 (s, 3H), 3.82 (s, 6H), 3.77 (s, 3H), 2.49-2.55 (m, 2H), 1.24 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.3, 156.1, 153.8, 142.5, 139.6, 137.0, 135.7, 119.2, 99.6, 61.0, 56.9, 53.2, 22.7, 12.4.

General procedure for the preparation of pyridazine derivatives 3.29 by amide coupling:

To a solution of **3.27** (0.5 mmol) in 5 mL of MeOH/water was added sodium hydroxyde (200 mg, 5 mmol) dissolved in water, the reaction was heated at 80 °C for 16 hours. After an acidic workup and evaporation to the carbolylic acid derivative **3.28**, the crude was used directly without further purification. To a solution of **3.28** in THF was added triethylamine (83 μL, 1.3 mmol) followed by TBTU (209 mg, 1.2 mmol) and an amine (0.75 mmol). 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the pyridazine **3.29**.

General procedure for the preparation of pyridazine derivatives 3.29 by Dabal-Me$_3$:

A solution of amine (1.5 mmol) and Dabal-Me_3 (385 mg, 1.5 mmol) in 5 mL of THF was heated at 40 °C for 1 hour, after which **3.27** (0.5 mmol) was added. The reaction mixture was refluxed until completion of the reaction (24-96 hours monitored by TLC). 15 mL of saturated aqueous NH_4Cl was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the pyridazine **3.29**.

4-(Ethylamino)-*N*-(3,4,5-trimethoxyphenyl)-6-phenylpyridazine-3-carboxamide (3.29a)

Brown solid, 37% yield; Data for **3.29a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.85 (br. s, NH), 8.58-8.62 (m, 1H), 8.20-8.24 (m, 2H), 7.55-7.5 (m, 3H), 7.40 (s, 2H), 7.37 (s, 1H), 3.79 (s, 6H), 3.66 (s, 3H), 3.42-3.50 (m, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.9, 158.4, 152.8, 147.0, 136.5, 135.5, 134.3, 130.2, 129.0, 127.4, 103.7, 98.4, 60.3, 56.0, 36.0, 14.0.

***N*-cyclohexyl-6-phenyl-4-(phenylamino)pyridazine-3-carboxamide (3.29h)**

Beige solid, 47% yield; Data for **3.29h**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.82 (br. s, NH), 9.12 (d, *J* = 8.5 Hz, NH), 7.99-8.01 (m, 2H), 7.52-7.54 (m, 4H), 7.46-7.49 (m, 2H), 7.42-7.44 (m, 2H), 7.24-7.26 (m, 1H), 3.85-3.90 (m, 1H), 1.60-1.87 (m, 5H), 1.15-1.55 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 158.7, 144.5, 137.7, 136.3, 130.2, 129.9, 129.0, 127.1, 125.3, 122.9, 104.5, 48.1, 32.0, 25.2, 24.9.

Morpholino-(6-phenyl-4-(phenylamino)pyridazin-3-yl)-methanone (3.29i)

Yellow solid, 50% yield; Data for **3.29i**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (br. s, NH), 7.93-7.95 (m, 2H), 7.49-7.52 (m, 3H), 7.42-7.45 (m, 3H), 7.35-7.36 (m, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 3.70-3.75 (m, 4H), 3.60-3.63 (m, 2H), 3.47-3.50 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.4, 157.9, 144.4, 141.9, 138.5, 136.6, 129.9, 129.6, 129.0, 127.0, 125.0, 123.1, 104.9, 66.4, 65.9, 47.2, 42.0.

***N*-(4-fluorophenyl)-6-phenyl-4-(phenylamino)pyridazine-3-carboxamide (3.29j)**

Brown solid, 47% yield; Data for **3.29j**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.26 (br. s, NH), 10.48 (br. s, NH), 8.02-8.04 (m, 2H), 7.94-7.97 (m, 2H), 7.53-7.57 (m, 4H), 7.46-7.51 (m, 4H), 7.23-7.29 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.0, 159.7, 158.8, 158.0, 144.8, 137.6, 136.5, 136.1, 134.4, 130.3, 129.9, 129.1, 127.2, 125.6, 1234, 123.2, 123.1, 115.4, 115.3, 104.8.

6-Phenyl-4-(phenylamino)-*N*-(pyridin-2-yl)pyridazine-3-carboxamide (3.29k)

Brown solid, 33% yield; Data for **3.29k**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 (br. s, NH), 10.34 (br. s, NH), 8.44-8.46 (m, 1H), 8.24 (d, *J* = 8.3 Hz, 1H), 8.01-8.04 (m, 2H), 7.92-7.95 (m, 1H), 7.53-7.56 (m, 4H), 7.48-7.51 (m, 4H), 7.29-7.31 (m, 1H), 7.24-7.27 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.7, 159.2, 150.4, 148.7, 145.2, 138.8, 137.2, 135.9, 135.4, 130.4, 130.0, 129.1, 127.3, 125.9, 123.6, 120., 113.9, 105.2.

6-Cyclohexyl-*N*-cyclopropyl-4-(phenylamino)pyridazine-3-carboxamide (3.29l)

Brown solid, 48% yield; Data for **3.29l**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.63 (br. s, NH), 9.25 (d, *J* = 4.7 Hz, 1H), 7.43-7.48 (m, 2H), 7.33 (d, *J* = 7.4 Hz, 2H), 7.22 (t, *J* = 7.3 Hz, 1H), 7.02 (s, 1H), 2.90-2.99 (m, 1H), 2.69-2.78 (m, 1H), 1.66-1.87 (m, 5H), 1.18-1.55 (m, 5H), 0.71-0.76 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.9, 167.8, 144.0, 137.8, 136.1, 129.9, 125.1, 122.8, 105.2, 43.9, 32.1, 25.9, 25.6, 22.8, 5.8.

(6-Cyclohexyl-4-(phenylamino)pyridazin-3-yl)-morpholino-methanone (3.29m)

Brown solid, 44% yield; Data for **3.29m**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (br. s, NH), 7.39-7.44 (m, 2H), 7.26 (d, *J* = 7.5 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.92 (s, 1H), 3.67-3.73 (m, 4H), 3.57-3.61 (m, 2H), 3.40-3.44 (m, 2H), 2.64-2.75 (m, 1H), 1.64-1.87 (m, 5H), 1.18-1.52 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6, 164.6, 144.1, 141.6, 138.7, 129.6, 124.7, 122.9, 105.6, 66.4, 66.0, 47.2, 44.1, 42.0, 32.1, 26.0, 25.6.

6-Cyclohexyl-*N*-(4-fluorophenyl)-4-(phenylamino)pyridazine-3-carboxamide (3.29n)

Brown solid, 39% yield; Data for **3.29n**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.14 (br. s, NH), 10.30 (br. s, NH), 7.90-7.92 (m, 2H), 7.45-7.48 (m, 2H), 7.36-7.37 (m, 2H), 7.21-7.26 (m, 3H), 7.06 (s, 1H), 2.76-2.81 (m, 1H), 1.69-1.89 (m, 5H), 1.20-1.56 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.1, 165.2, 144.5, 137.6, 136.2, 134.3, 129.9, 125.4, 123.1, 123.0, 122.9, 115.4, 115.2, 105.6, 43.9, 32.0, 25.9, 25.5.

6-Cyclohexyl-4-(phenylamino)-*N*-(pyridin-2-yl)pyridazine-3-carboxamide (3.29o)

Beige solid, 31% yield; Data for **3.29o**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (br. s, NH), 10.20 (br. s, NH), 8.42-8.44 (m, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.90-7.94 (m, 1H), 7.47-7.50 (m, 2H), 7.37-7.40 (m, 2H), 7.23-7.29 (m, 2H), 7.06 (s, 1H), 2.77-2.82 (m, 1H), 1.67-1.89 (m, 5H) 1.19-1.55 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.7, 164.9, 150.4, 148.7, 145.0, 138.8, 137.4, 135.1, 129.9, 125.7, 123.5, 120.6, 113.8, 106.0, 44.0, 32.0, 25.9, 25.5.

4-(3,4,5-Trimethoxyphenylamino)-6-ethyl-*N*-methyl-pyridazine-3-carboxamide (3.29p)

Pale yellow solid, 60% yield; Data for **3.29p**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.52 (br. s, NH), 9.24-9.29 (m, 1H), 7.13 (s, 1H), 6.63 (s, 2H), 3.78 (s, 6H), 3.67 (s, 3H), 2.85 (d, *J* = 4.8 Hz, 3H), 2.80 (q, *J* = 7.6 Hz, 2H), 1.21 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.0, 165.6, 165.3, 153.7, 144.4, 135.9, 134.9, 133.5, 106.5, 100.9, 60.4, 56.0, 28.7, 25.9, 13.7.

(4-(3,4,5-Trimethoxyphenylamino)-6-ethylpyridazin-3-yl)-morpholino-methanone (3.29q)

Yellow solid, 56% yield; Data for **3.29q**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (br. s, NH), 7.06 (s, 1H), 6.58 (s, 2H), 3.76 (s, 6H), 3.70-3.73 (m, 4H), 3.66 (s, 3H), 3.57-3.59 (m, 2H), 3.41-3.43 (m, 2H), 2.75 (q, *J* = 7.6 Hz, 2H), 1.20 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.6, 164.0, 153.4, 143.7, 141.7, 134.6, 134.3, 106.7, 100.7, 66.4, 66.0, 60.2, 56.0, 47.2, 42.0, 28.6, 13.7.

4-(3,4,5-Trimethoxyphenylamino)-*N*-(4-fluorophenyl)-6-phenylpyridazine-3-carboxamide (3.29s)

Beige solid, 35% yield; Data for **3.29s**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.25 (br. s, NH), 10.35 (br. s, NH), 8.04-8.08 (m, 2H), 7.94-7.98 (m, 2H), 7.63 (s, 1H), 7.53-7.57 (m, 3H), 7.25 (t, *J* = 8.9 Hz, 2H), 6.80 (s, 2H), 3.80 (s, 6H), 3.70 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.1, 158.7, 153.8, 145.3, 136.3, 135.5, 134.9, 134.5, 133.1, 130.4, 129.2, 127.2, 123.2, 115.5, 115.2, 105.3, 101.6, 60.3, 56.3.

Methyl 6-isopropyl-4-phenylpyridazine-3-carboxylate (3.30)

3.24b (196 mg, 1.0 mmol) was dissolved in 5 mL of phosphorus oxychloride at room temperature. The reaction mixture was heated at 95 °C for 4 hours after which the color of the solution became brown. The flask was allowed to cool to room temperature and the excess of phosphorus oxychloride was concentrated in vacuo to a brown oil. The oil was dissolved in 15 mL of EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$, the organic layer was dried over Na_2SO_4 and concentrated to afford crude **3.26b** as brown oil used directly without further purification.

To a solution of **3.26b** in 5 mL of dioxane under argon was added phenyl boronic acid (146 mg, 1.2 mmol), K_3PO_4 (467 mg, 2.2 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction for 48 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:1) to afford the aryl substituted pyridazine **3.30** in 34% yield as beige solid. Data for **3.30**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.35 (m, 3H), 7.26-7.28 (m, 3H), 3.71 (s, 3H), 3.27-3.33 (m, 1H), 1.30 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.1, 160.7, 154.9, 145.6, 137.0, 129.8, 127.5, 126.3, 117.2, 55.6, 31.7, 22.4.

Pyridazines 3.31a and 3.31b were synthesized following the method with Dabal-Me_3 applied to 3.29.

***N*-(4-fluorophenyl)-6-isopropyl-4-phenylpyridazine-3-carboxamide (3.31a)**

Beige solid, 38% yield; Data for **3.31a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (br. s, NH), 7.44-7.47 (m, 2H), 7.37-7.41 (m, 3H), 7.29-7.32 (m, 3H), 7.24-7.27 (m, 2H), 3.19-3.24 (m, 1H), 1.32 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 158.3, 157.7, 144.8, 137.9, 134.1, 129.0, 127.3, 126.5, 124.8, 121.6, 116.7, 31.5, 21.9.

General procedure for the preparation of β-hydroxy ketone derivatives 3.34 by organocatalysis:

To a solution of aldehyde **3.33** (1 mmol) in a mixture of DMSO (4 mL) and acetone (1 mL) was added L-proline (29 mg, 0.25 mmol). The resulting mixture was stirred under argon at room temperature for 5 hours. The reaction mixture was treated with 10 mL of saturated aqueous NH_4Cl, the layers were separated, and the aqueous layer was extracted several times with EtOAc, dried over Na_2SO_4, and concentrated in vacuo. A purification by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) afforded the desired aldol product **3.34**.

4-Hydroxy-(4-nitrophenyl)-2-butanone (3.34a)

Pink solid, 57% yield; Data for **3.34a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 5.70 (d, *J* = 4.8 Hz, 1H), 5.13-5.16 (m, 1H), 2.75 (d, *J* = 6.6 Hz, 2H), 2.14 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 206.3, 153.3, 146.5, 127.0, 123.4, 68.1, 52.4, 30.4; HRMS calcd for $C_{10}H_{11}NO_4$ $[M+Na]^+$ 232.0581, found 232.0584.

(4-Cyanophenyl)-4-hydroxy-2-butanone (3.34b)

Beige solid, 55% yield; Data for **3.34b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.82 (d, *J* = 8.1 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 5.65 (d, *J* = 4.7 Hz, 1H), 5.10-5.13 (m, 1H), 2.76 (d, *J* = 5.8 Hz, 2H), 2.16 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 151.1, 132.2, 126.8, 119.0, 109.7, 68.3, 52.4, 30.4; HRMS calcd for $C_{11}H_{11}NO_2$ $[M+Na]^+$ 212.0682, found 212.0677.

General procedure for the preparation of β-hydroxy ketone derivatives 3.37 by Mukaiyama aldol reaction:

To a solution of ketone **3.36** (1 mmol) in 10 mL of THF under argon at -78 °C was slowly added a solution of 1 M LiHMDS (1.2 mL, 1.2 mmol) and the reaction mixture was stirred for 1 hour at -78 °C, prior to the addition of chlorotrimethylsilane (153 μL, 1.2 mmol) in THF. The mixture was further stirred for 1 hour, then allowed to warm up to room temperature. The reaction was quenched with 10 mL of saturated aqueous NH_4Cl, after addition of EtOAc, the layers were separated, and the aqueous layer was extracted several times with EtOAc, dried over Na_2SO_4, and concentrated in vacuo to afford the corresponding silyl enol ether used directly without further purification for the next step.

To a solution of silyl enol ether in 10 mL of CH_2Cl_2 under argon at -78 °C was added acetaldehyde (85 μL, 1.5 mmol) for **3.37a** or propionaldehyde for **3.37b** (110 μL, 1.5 mmol) followed by a slow addition of $TiCl_4$ (121 μL, 1.1 mmol). The resulting red solution was stirred at -78 °C for 5 hours and then the reaction was quenched with 10 mL of saturated aqueous NH_4Cl and warmed to room temperature. The organic layer was separated and then washed with 15 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted with 20 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to obtain the desired β-hydroxy ketone **3.37**.

2-Hydroxy-(thiophen-2-yl)-4-butanone (3.37a)

Colorless oil, 63% yield; Data for **3.37a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (d, *J* = 4.9 Hz, 1H), 7.94 (d, *J* = 3.8 Hz, 1H), 7.24 (t, *J* = 4.8 Hz, 1H), 4.70 (d, *J* = 4.9 Hz, 1H), 4.15-4.17 (m, 1H), 2.85-3.09 (m, 2H), 1.14 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.1, 144.7, 134.9, 133.6, 128.8, 63.8, 48.4, 23.8; HRMS calcd for $C_8H_{10}N_2S$ $[M+Na]^+$ 193.0294, found 193.0297.

(4-Chlorophenyl)-3-hydroxy-5-pentanone (3.37b)

Yellow oil, 68% yield; Data for **3.37b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (d, *J* = 8.5 Hz, 2H), 7.58 (d, *J* = 8.5 Hz, 2H), 4.62 (d, *J* = 5.5 Hz, 1H), 3.89-3.95 (m, 1H), 3.90-3.94 (m, 1H), 2.94-3.10 (m, 2H), 1.40-1.48 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.5, 138.0, 136.0, 130.1, 128.8, 68.6, 45.9, 30.2, 10.0; HRMS calcd for $C_{11}H_{13}ClO_2$ $[M+Na]^+$ 235.0497, found 235.0502.

General procedure for the preparation of α-diazo-1,3-diketone 3.35a, 3.35b, 3.38a and 3.38b:

To a solution of β-hydroxy ketone (**3.34a**, **3.34b**, **3.37a**, **3.37b**) (1 mmol) in 10 mL of acetonitrile under argon at 0 °C was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was allowed to warm to room temperature and then filtered. The filtrate was concentrated to afford the 1,3-diketone used directly without further purification for the next step.

To a solution of 1,3-diketone in 15 mL of acetonitrile under argon at 0 °C was added successively triethylamine (181 μL, 1.3 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (240 mg, 1 mmol). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with a mixture of diethyl ether/hexane (1/1, 30 mL) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:4) to afford the α-diazo-1,3-diketone **3.35a**, **3.35b**, **3.38a** and **3.38b**.

3-Diazo-(4-nitrophenyl)-butan-2,4-dione (3.35a)

Yellow solid, 85% yield; Data for **3.35a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.7 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 188.8, 184.0, 149.3, 142.9, 129.1, 123.9, 84.8, 28.7.

(4-Cyanophenyl)-3-diazo-butan-2,4-dione (3.35b)

Beige solid, 77% yield; Data for **3.35b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.01 (d, *J* = 8.2 Hz, 2H), 7.87 (d, *J* = 8.3 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.0, 184.2, 141.4, 132.8, 128.4, 118.2, 114.4, 84.6, 28.7.

3-Diazo-(thiophen-2-yl)-butan-2,4-dione (3.38a)

Yellow oil, 87% yield; Data for **3.38a**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69-7.70 (m, 1H), 7.60-7.61 (m, 1H), 7.15-7.16 (m, 1H), 2.60 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.8, 175.1, 141.9, 133.5, 130.4, 127.9, 81.7, 29.3.

(4-Chlorophenyl)-4-diazo-pentan-3,5-dione (3.38b)

Pale yellow solid, 81% yield; Data for **3.38b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 2.86 (q, *J* = 7.3 Hz, 2H), 1.05 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.6, 184.1, 137.2, 136.4, 129.6, 128.8, 83.2, 33.8, 8.2.

3-Diazo-(phenyl)-butan-2,4-dione (3.40)

To a solution of compound **3.39** (3 g, 18.50 mmol) in 50 mL of acetonitrile under argon at 0 °C was added successively triethylamine (3.3 mL, 24.05 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (4.4 g, 18.50 mmol). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 100 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was

concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the desired compound **3.40** in 89% yield as yellow solid; Data for **3.40**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, *J* = 7.2 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.52-7.55 (m, 2H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.8, 185.0, 137.5, 132.5, 128.8, 127.6, 83.6, 28.8.

General procedure for the preparation of 3.41 by titanium aldol reaction:

To a solution of α-diazo-1,3-diketone (**3.35a**, **3.35b**, **3.38a**, **3.38b** and **3.40**) (1 mmol) in 10 mL of CH_2Cl_2 under argon at -78 °C was added dropwise $TiCl_4$ (121 μL, 1.1 mmol) followed by Et_3N (153 μL, 1.1 mmol). The resulting red solution was stirred at -78 °C for 1 hour, after which a solution of aldehyde (0.9 mmol) in CH_2Cl_2 was slowly added. The reaction mixture was stirred at -78 °C for 4 hours and then the reaction was quenched with 10 mL of saturated aqueous NH_4Cl and warmed to room temperature. The organic layer was separated and then washed with 15 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted with 20 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the aldol product **3.41**.

6-Diazo-3-hydroxy-(4-nitrophenyl)-heptan-5,7-dione (3.41a)

Yellow oil, 69% yield; Data for **3.41a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (d, *J* = 8.1 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 4.75-4.77 (m, 1H), 3.82-3.87 (m, 1H), 2.86-2.91 (m, 2H), 1.37-1.47 (m, 2H), 0.88 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.0, 184.3, 149.3, 143.1, 129.1, 123.8, 85.2, 68.4, 47.9, 30.0, 10.0; HRMS calcd for $C_{13}H_{13}N_3O_5$ $[M+Na]^+$ 314.0748, found 314.0752.

(4-Cyanophenyl)-6-diazo-3-hydroxy-heptan-5,7-dione (3.41b)

Yellow oil, 81% yield; Data for **3.41b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, *J* = 8.1 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 2H), 4.73-4.75 (m, 1H), 3.85-3.87 (m, 1H), 2.83-2.93 (m, 2H), 1.30-1.46 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.1, 184.5, 141.5, 132.7, 128.4, 118.2, 114.4, 84.8, 68.4, 47.9, 30.0, 10.0; HRMS calcd for $C_{14}H_{13}N_3O_3$ $[M+Na]^+$ 294.0849, found 294.0849.

(4-Cyanophenyl)-4-diazo-1-(2-fluorophenyl)-1-hydroxy-pentan-3,5-dione (3.41c)

Yellow oil, 50% yield; Data for **3.41c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.99 (d, *J* = 8.6 Hz, 2H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.52-7.56 (m, 1H), 7.29-7.34 (m, 1H), 7.20-7.23 (m, 1H), 7.12-7.16 (m, 1H), 5.63 (d, *J* = 4.9 Hz, 1H), 5.34-5.38 (m, 1H), 3.36 (m, 1H), 3.01 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.0, 184.4, 160.0, 158.0, 141.3, 132.8, 131.7, 129.0, 128.4, 127.9, 124.5, 118.2, 115.1, 114.4, 84.8, 62.8, 48.5; HRMS calcd for $C_{18}H_{12}FN_3O_3$ $[M+Na]^+$ 360.0755, found 360.0761.

6-Diazo-3-hydroxy-(thiophen-2-yl)-heptan-5,7-dione (3.41d)

Yellow oil, 77% yield; Data for **3.41d**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04-8.05 (m, 1H), 7.92-7.93 (m, 1H), 7.23-7.25 (m, 1H), 4.66 (d, *J* = 5.7 Hz, 1H), 3.84-3.88 (m, 1H), 2.91-2.98 (m, 2H), 1.33-1.48 (m,

2H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.4, 141.8, 134.6, 132.0, 128.4, 82.4, 68.3, 48.0, 30.0, 10.0; HRMS calcd for $C_{11}H_{12}N_2O_3S$ $[M+Na]^+$ 275.0461, found 275.0468.

(4-Chlorophenyl)-6-diazo-3-hydroxy-4-methyl-heptan-5,7-dione (3.41e)

Yellow oil, 63% yield; Inseparable diastereomeric mixture; Data for **3.41e** (major isomer): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 4.60-4.67 (m, 1H), 4.18 (d, *J* = 4.8 Hz, 1H), 3.10-3.16 (m, 1H), 1.63-1.72 (m, 2H), 0.98-1.20 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 171.6, 140.3, 133.5, 130.5, 128.8, 84.3, 57.4, 36.3, 23.0, 13.2, 10.0; HRMS calcd for $C_{14}H_{15}ClN_2O_3$ $[M+Na]^+$ 317.0664, found 317.0657.

4-Diazo-1-hydroxy-(phenyl)-1-(thiophen-2-yl)-pentan-3,5-dione (3.41f)

Yellow oil, 57% yield; Data for **3.41f**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (m, 2H), 7.60 (m, 1H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.41 (dd, *J* = 4.9, 1.3 Hz, 1H), 6.97-7.02 (m, 2H), 5.88-5.90 (m, 1H), 5.38-5.40 (m, 1H), 3.24-3.46 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.7, 185.1, 149.7, 137.5, 132.6, 128.8, 127.7, 126.8, 124.5, 123.2, 83.9, 65.2, 50.4; HRMS calcd for $C_{15}H_{12}N_2O_3S$ $[M+Na]^+$ 323.0461, found 323.0460.

4-Diazo-1-hydroxy-(phenyl)-1-(phenyl)-pentan-3,5-dione (3.41g)

Yellow oil, 52% yield; Data for **3.41g**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.46-7.60 (m, 3H), 7.4-7.44 (m, 4H), 7.28-7.42 (m, 3H), 5.28 (m, 1H), 3.56 (m, 1H), 3.33-3.44 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.4, 184.7, 142.6, 136.7, 132.6, 128.7, 128.2, 127.4, 127.1, 125.5, 83.6, 70.3, 49.4; HRMS calcd for $C_{17}H_{14}N_2O_3$ $[M+Na]^+$ 317.0897, found 317.0901.

4-Diazo-1-(cyclohexyl)-1-hydroxy-(phenyl)-pentan-3,5-dione (3.41h)

Yellow oil, 79% yield; Data for **3.41h**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, *J* = 7.3 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 4.61 (d, *J* = 5.8 Hz, 1H), 3.74-3.78 (m, 1H), 2.90 (d, *J* = 5.7 Hz, 2H), 1.59-1.76 (m, 5H), 0.95-1.28 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 191.4, 185.2, 137.6, 132.5, 128.7, 127.7, 83.6, 71.0, 45.5, 43.7, 28.8, 27.6, 26.2, 25.9, 25.8; HRMS calcd for $C_{17}H_{20}N_2O_3$ $[M+Na]^+$ 323.1366, found 323.1363.

6-Diazo-3-hydroxy-2-methyl-(phenyl)-heptan-5,7-dione (3.41i)

Yellow oil, 85% yield; Data for **3.41i**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, *J* = 7.3 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 4.65 (br. s, 1H), 3.76-3.78 (m, 1H), 2.85-2.95 (m, 2H), 1.60-1.64 (m, 1H), 0.85 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 191.3, 185.2, 137.6, 132.5, 128.7, 127.7, 83.7, 71.5, 45.4, 33.6, 18.7, 17.5; HRMS calcd for $C_{14}H_{16}N_2O_3$ $[M+Na]^+$ 283.1053, found 283.1052.

General procedure for the preparation of pyridazine derivatives 3.42:

To a solution of **3.41** (1 mmol) in 10 mL of acetonitrile under argon was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was

allowed to warm to room temperature and then filtered. The filtrate was concentrated and used directly without further purification for the next step.

To the previously prepared crude in 15 mL of *i*-Pr_2O was added P(*n*-Bu)$_3$ (250 μL, 1.0 mmol). The reaction mixture was stirred under argon at room temperature for 30 minutes, which after this time a precipitate was formed. The suspension was filtered, washed with *i*-Pr_2O and dried to afford **3.42a**, **3.42b**, **3.42d**, **3.42e** and **3.42i**. **3.42c**, **3.42f**, **3.42g** and **3.42h** were purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 8:2) due to no formation of precipitate.

(6-Ethyl-4-hydroxy-pyridazinyl)-(4-nitrophenyl)-methanone (3.42a)

Brown solid, 60% yield; Data for **3.42a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.54 (br. s, 1H), 8.34 (d, *J* = 8.5 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 6.47 (s, 1H), 2.63 (q, *J* = 7.4 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.0, 152.7, 150.4, 140.2, 130.6, 124.1, 116.5, 24.3, 12.5; HRMS calcd for $C_{13}H_{11}N_3O_4$ $[M+H]^+$ 274.0822, found 274.0829.

(4-Cyanophenyl)-(6-ethyl-4-hydroxy-pyridazinyl)-methanone (3.42b)

Yellow solid, 57% yield; Data for **3.42b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.55 (br. s, 1H), 8.02 (d, *J* = 8.3 Hz, 2H), 7.96 (d, *J* = 8.3 Hz, 2H), 6.46 (s, 1H), 2.62 (q, *J* = 7.6 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.9, 169.1, 156.8, 152.8, 138.7, 133.0, 129.8, 118.1, 116.3, 115.9, 24.1, 12.5; HRMS calcd for $C_{14}H_{11}N_3O_2$ $[M+H]^+$ 254.0924, found 254.0928.

(4-Cyanophenyl)-(6-(2-fluorophenyl)-4-hydroxy-pyridazinyl)-methanone (3.42c)

Beige solid, 45% yield; Data for **3.42c**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.94 (br. s, 1H), 8.05 (m, 4H), 7.73-7.76 (m, 1H), 7.66-7.69 (m, 1H), 7.46-7.49 (m, 1H), 7.43-7.45 (m, 1H), 6.78 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.7, 159.9, 158.3, 152.9, 138.7, 133.4, 133.3, 133.0, 130.9, 130.0, 125.9, 118.9, 118.2, 116.6, 116.5, 116.0; HRMS calcd for $C_{18}H_{10}FN_3O_2$ $[M+H]^+$ 320.0830, found 320.0831.

(6-Ethyl-4-hydroxy-pyridazinyl)-(thiophen-2-yl)-methanone (3.42d)

Orange solid, 55% yield; Data for **3.42d**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.41 (br. s, 1H), 8.13-8.14 (m, 1H), 7.69-7.70 (m, 1H), 7.24-7.26 (m, 1H), 6.42 (s, 1H), 2.61 (q, *J* = 7.6 Hz, 2H), 1.23 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 184.0, 169.1, 156.5, 153.0, 142.2, 136.8, 136.5, 129.1, 116.0, 24.1, 12.5; HRMS calcd for $C_{11}H_{10}N_2O_2S$ $[M+H]^+$ 235.0536, found 235.0540.

(4-Chlorophenyl)-(6-ethyl-4-hydroxy-5-methyl-pyridazinyl)-methanone (3.42e)

Colorless oil, 57% yield; Data for **3.42e**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.37 (br. s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 2.69 (q, *J* = 7.3 Hz, 2H), 1.95 (s, 3H), 1.22 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.9, 168.6, 153.1, 150.6, 139.0, 134.4, 131.2, 129.1, 125.5, 22.6, 12.7, 9.1; HRMS calcd for $C_{14}H_{13}ClN_2O_2$ $[M+H]^+$ 277.0738, found 277.0739.

(4-Hydroxy-6-(thiophen-2-yl)-pyridazinyl)-(phenyl)-methanone (3.42f)

Orange solid, 53% yield; Data for **3.42f**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88-7.92 (m, 4H), 7.72 (t, *J* = 7.4 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 2H), 7.29-7.31 (m, 1H), 6.94 (br. s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 192.4, 135.5, 134.3, 131.0, 129.6, 129.0, 128.9; HRMS calcd for $C_{15}H_{10}N_2O_2S$ $[M+H]^+$ 283.0536, found 283.0534.

(4-Hydroxy-6-phenyl-pyridazinyl)-(phenyl)-methanone (3.42g)

Brown solid, 48% yield; Data for **3.42g**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.72 (t, *J* = 7.4 Hz, 1H), 7.56-7.62 (m, 5H), 6.85 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 192.6, 135.4, 134.3, 131.2, 129.5, 129.3, 129.0, 127.4, 115.3; HRMS calcd for $C_{17}H_{12}N_2O_2$ $[M+H]^+$ 277.0971, found 277.0978.

(6-Cyclohexyl-4-hydroxy-pyridazinyl)-(phenyl)-methanone (3.42h)

Beige solid, 52% yield; Data for **3.42h**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.36 (br. s, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.55 (t, *J* = 8.1 Hz, 2H), 6.40 (s, 1H), 2.54-2.59 (m, 1H), 1.69-1.90 (m, 5H), 1.33-1.52 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 192.7, 159.5, 154.1, 135.4, 134.3, 129.4, 129.0, 114.2, 40.1, 31.0, 25.7, 25.1; HRMS calcd for $C_{17}H_{18}N_2O_2$ $[M+H]^+$ 283.1441, found 283.1440.

(4-Hydroxy-6-isopropyl-pyridazinyl)-(phenyl)-methanone (3.42i)

Beige solid, 55% yield; Data for **3.42i**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.41 (br. s, 1H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 2H), 6.43 (s, 1H), 2.88-2.93 (m, 1H), 1.27 (d, *J* = 7.1 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 192.7, 169.3, 160.5, 154.2, 135.4, 134.3, 129.4, 129.0, 113.8, 30.3, 21.1; HRMS calcd for $C_{14}H_{14}N_2O_2$ $[M+H]^+$ 243.1128, found 243.1130.

2-Diazo-(4-methoxyphenyl)-1-(thiophen-2-yl)-pent-4-ene-1,3-dione (3.43)

Yellow solid, 62% yield; Data for **3.43**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (d, *J* = 3.9 Hz, 1H), 7.97 (d, *J* = 3.8 Hz, 1H), 7.56-7.74 (m, 4H), 7.26 (t, *J* = 3.9 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 181.2, 175.6, 161.6, 142.1, 141.8, 134.7, 132.0, 130.6, 128.4, 126.9, 120.5, 114.7, 82.9, 55.5.

Method A: preparation of pyrazolo[4,3-c]pyridazine analogues 3.44:

A solution of **3.42** (1 mmol) in 5 mL of $POCl_3$ was heated at 95 °C for 4 hours, after which the reaction mixture was cooled down to room temperature. $POCl_3$ was evaporated and the residue was dissolved in EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford the chlorinated pyridazine used directly without further purification for the next step.

To a solution of chlorinated pyridazine in 5 mL of EtOH was added hydrazine hydrate 64% (63 μL, 1.3 mmol) followed by Et_3N (181 μL, 1.3 mmol) and refluxed for 4 hours. After completion of the reaction

(monitored by TLC), the reaction mixture was allowed to cool to room temperature. CH_2Cl_2 was added and the organic layer was then washed 2 times with 10 mL of saturated aqueous $NaHCO_3$, and 1 time with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (EtOAc) to obtain the desired pyrazolo[4,3-c]pyridazine **3.44**.

3-(4-Cyanophenyl)-6-ethyl-pyrazolo[4,3-c]pyridazine (3.44a)

Beige solid, 83% yield; Data for **3.44a**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (d, *J* = 8.5 Hz, 2H), 8.05 (d, *J* = 8.5 Hz, 2H), 7.80 (s, 1H), 3.16 (q, *J* = 7.6 Hz, 2H), 1.38 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.0, 144.1, 141.0, 135.9, 133.8, 133.0, 127.3, 118.9, 111.1, 105.2, 28.9, 14.6; HRMS calcd for $C_{14}H_{11}N_5$ $[M+H]^+$ 250.1087, found 250.1089.

3-(4-Cyanophenyl)-6-(2-fluorophenyl)-pyrazolo[4,3-c]pyridazine (3.44b)

White solid, 79% yield; Data for **3.44b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.25 (br. s, 1H), 8.78 (d, *J* = 8.3 Hz, 2H), 8.25 (s, 1H), 8.05-8.14 (m, 3H), 7.59-7.61 (m, 1H), 7.45 (t, *J* = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.7, 158.4, 149.6, 144.2, 141.3, 135.7, 133.3, 133.1, 131.7, 127.4, 125.1, 118.9, 116.7, 116.4, 111.4, 107.8; HRMS calcd for $C_{18}H_{10}N_5$ $[M+H]^+$ 316.0993, found 316.0989.

3-(4-Chlorophenyl)-7-methyl-6-ethyl-pyrazolo[4,3-c]pyridazine (3.44c)

Beige solid, 87% yield; Data for **3.44c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (d, *J* = 8.6 Hz, 2H), 7.68 (d, *J* = 8.6 Hz, 2H), 3.17 (q, *J* = 7.5 Hz, 2H), 2.61 (s, 3H), 1.37 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.2, 143.6, 141.6, 134.7, 133.5, 130.6, 129.1, 128.4, 115.9, 25.6, 13.9, 12.0; HRMS calcd for $C_{14}H_{13}ClN_4$ $[M+H]^+$ 273.0901, found 273.0905.

3-Phenyl-6-(thiophen-2-yl)-pyrazolo[4,3-c]pyridazine (3.44d)

Pale yellow solid, 81% yield; Data for **3.44d**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.94 (br. s, 1H), 8.59 (d, *J* = 8.4 Hz, 2H), 8.35 (s, 1H), 8.06 (d, *J* = 3.7 Hz, 1H), 7.76 (d, *J* = 5.0 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 2H), 7.50 (m, 1H), 7.26 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 148.9, 144.5, 143.2, 141.2, 133.5, 131.3, 129.4, 129.2, 129.0, 128.5, 127.0, 126.5, 101.3; HRMS calcd for $C_{15}H_{10}N_4S$ $[M+H]^+$ 279.0699, found 279.0701.

3-Phenyl-6-phenyl-pyrazolo[4,3-c]pyridazine (3.44e)

Beige solid, 79% yield; Data for **3.44e**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.96 (br. s, 1H), 8.63 (d, *J* = 7.3 Hz, 2H), 8.34 (s, 1H), 8.30 (d, *J* = 7.4 Hz, 2H), 7.59-7.62 (m, 4H), 7.49-7.56 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 152.7, 144.6, 143.0, 137.0, 133.8, 131.4, 129.6, 129.2, 129.1, 129.0, 127.5, 127.0, 103.7; HRMS calcd for $C_{17}H_{12}N_4$ $[M+H]^+$ 273.1135, found 273.1133.

6-Isopropyl-3-phenyl-pyrazolo[4,3-c]pyridazine (3.44f)

Beige solid, 75% yield; Data for **3.44f**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.70 (br. s, 1H), 8.58 (d, *J* = 8.3 Hz, 2H), 7.71 (s, 1H), 7.58 (t, *J* = 7.4 Hz, 2H), 7.48 (t, *J* = 7.4 Hz, 1H), 3.46-3.51 (m, 1H), 1.41 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.2, 144.3, 142.7, 133.6, 131.6, 129.0, 128.9, 126.9, 103.5, 34.4, 22.9; HRMS calcd for $C_{14}H_{14}N_4$ $[M+H]^+$ 239.1291, found 239.1293.

Method B: preparation of thieno[3,2-c]pyridazine analogues 3.45:

A solution of **3.42** (1 mmol) in 5 mL of $POCl_3$ was heated at 95 °C for 4 hours, after which the reaction mixture was cooled down to room temperature. $POCl_3$ was evaporated and the residue was dissolved in EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford the chlorinated pyridazine used directly without further purification for the next step.

To a solution of chlorinated pyridazine in 5 mL of EtOH was added methyl thioglycolate (134 μL, 1.5 mmol) followed by Et_3N (417 μL, 3 mmol) and refluxed for 16 hours. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. CH_2Cl_2 was added and the organic layer was then washed 2 times with 10 mL of saturated aqueous $NaHCO_3$, and 1 time with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:9) to obtain the desired thieno[3,2-c]pyridazine **3.45**.

Ethyl 3-ethyl-7-(4-nitrophenyl)-thieno[3,2-c]pyridazine-6-carboxylate (3.45a)

Brown solid, 52% yield; Data for **3.45a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.36 (d, *J* = 8.8 Hz, 2H), 7.89 (d, *J* = 8.9 Hz, 2H), 4.25 (q, *J* = 7.0 Hz, 2H), 3.12 (q, *J* = 7.6 Hz, 2H), 1.36 (t, *J* = 7.6 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.1, 159.6, 156.4, 147.5, 139.4, 139.0, 138.0, 134.4, 132.3, 122.7, 119.6, 62.3, 28.8, 14.2, 13.8; HRMS calcd for $C_{17}H_{15}N_3O_4S$ $[M+H]^+$ 358.0856, found 358.0860.

Ethyl 3-ethyl-7-(thiophen-2-yl)-thieno[3,2-c]pyridazine-6-carboxylate (3.45b)

Beige solid, 38% yield; Data for **3.45b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81-7.83 (m, 2H), 7.58-7.59 (m, 1H), 7.19-7.21 (m, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.20 (q, *J* = 7.7 Hz, 2H), 1.47 (t, *J* = 7.7 Hz, 3H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.8, 159.6, 156.3, 137.9, 134.5, 131.7, 131.5, 131.0, 128.7, 126.5, 118.1, 62.3, 29.4, 14.0; HRMS calcd for $C_{15}H_{14}N_2O_2S_2$ $[M+H]^+$ 319.0569, found 319.0579.

Ethyl 7-phenyl-3-(thiophen-2-yl)-thieno[3,2-c]pyridazine-6-carboxylate (3.45c)

Yellow solid, 33% yield; Data for **3.45c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (s, 1H), 7.97 (d, *J* = 3.4 Hz, 1H), 7.81 (d, *J* = 4.8 Hz, 1H), 7.59-7.61 (m, 2H), 7.51-7.53 (m, 3H), 7.29 (d, *J* = 4.6 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 1.15 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4, 156.9, 149.5, 141.5,

140.4, 138.4, 133.5, 132.0, 130.8, 130.4, 128.9, 128.7, 127.7, 127.3, 115.8, 62.1, 13.8; HRMS calcd for $C_{19}H_{14}N_2O_2S_2$ $[M+H]^+$ 367.0569, found 367.0570.

Methyl 3-phenyl-7-phenyl-thieno[3,2-c]pyridazine-6-carboxylate (3.45d)

Brown solid, 41% yield; Data for **3.45d**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.25 (d, *J* = 7.3 Hz, 2H), 7.60-7.64 (m, 4H), 7.53-7.57 (m, 4H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 157.0, 153.2, 141.6, 138.5, 136.1, 133.1, 132.0, 130.8, 130.2, 129.3, 128.7, 127.7, 127.3, 117.9, 53.1; HRMS calcd for $C_{20}H_{14}N_2O_2S$ $[M+H]^+$ 347.0849, found 347.0846.

Method C: preparation of dihydro-pyridazino[4,3-e][1,4]diazepine analogues 3.46:

A solution of **3.42** (1 mmol) in 5 mL of $POCl_3$ was heated at 95 °C for 4 hours, after which the reaction mixture was cooled down to room temperature. $POCl_3$ was evaporated and the residue was dissolved in EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford the chlorinated pyridazine used directly without further purification for the next step.

To a solution of chlorinated pyridazine in 5 mL of EtOH was added ethylene diamine (87 µL, 1.3 mmol) followed by Et_3N (181 µL, 1.3 mmol) and refluxed for 16 hours. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. CH_2Cl_2 was added and the organic layer was then washed 2 times with 10 mL of saturated aqueous $NaHCO_3$, and 1 time with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (MeOH/EtOAc = 1:9) to obtain the desired dihydro-pyridazino[4,3-e][1,4]diazepine **3.46**.

3-Ethyl-6,7-dihydro-9-(4-nitrophenyl)-5H-pyridazino[4,3-e][1,4]diazepine (3.46a)

Pale yellow solid, 77% yield; Data for **3.46a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, *J* = 8.8 Hz, 2H), 7.65 (m, 3H), 6.72 (s, 1H), 4.10-4.11 (m, 2H), 3.51-3.52 (m, 2H), 2.75 (q, *J* = 7.6 Hz, 2H), 1.23 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.0, 161.7, 148.6, 147.3, 146.6, 139.8, 130.3, 122.7, 109.9, 53.3, 47.5, 28.0, 13.4; HRMS calcd for $C_{15}H_{15}N_5O_2$ $[M+H]^+$ 298.1298, found 298.1306.

9-(4-Cyanophenyl)-6,7-dihydro-3-(2-fluorophenyl)-5H-pyridazino[4,3-e][1,4]diazepine (3.46b)

White solid, 79% yield; Data for **3.46b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02-8.05 (m, 1H), 7.96-7.97 (m, 1H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.64 (d, *J* = 7.8 Hz, 2H), 7.53-7.57 (m, 1H), 7.36-7.41 (m, 2H), 7.32 (s, 1H), 4.16-4.17 (m, 2H), 3.56-3.57 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.0, 161.1, 159.1, 151.8, 146.5, 131.8, 131.7, 131.5, 130.7, 130.0, 125.0, 124.3, 118.9, 116.5, 112.3, 111.0, 53.4, 47.6; HRMS calcd for $C_{20}H_{14}FN_5$ $[M+H]^+$ 344.1306, found 344.1301.

3-Ethyl-6,7-dihydro-9-(thiophen-2-yl)-5H-pyridazino[4,3-e][1,4]diazepine (3.46c)

Yellow solid, 81% yield; Data for **3.46c**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.58 (d, *J* = 4.9 Hz, 1H), 7.36 (d, *J* = 3.0 Hz, 1H), 7.31 (br. s, 1H), 7.05 (t, *J* = 4.0 Hz, 1H), 6.69 (s, 1H), 3.98-3.99 (m, 2H), 3.52-3.53 (m, 2H), 2.78 (q, *J* = 7.6 Hz, 2H), 1.26 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.6, 162.1, 146.0, 145.1, 139.9, 130.5, 129.2, 127.2, 110.2, 51.4, 48.3, 28.0, 13.4; HRMS calcd for $C_{13}H_{14}N_4S$ $[M+H]^+$ 259.1012, found 259.1007.

9-(4-Chlorophenyl)-3-Ethyl-6,7-dihydro-4-methyl-5H-pyridazino[4,3-e][1,4]diazepine (3.46d)

Beige solid, 82% yield; Data for **3.46d**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 (m, 4H), 6.64 (br. s, 1H), 4.01-4.04 (m, 2H), 3.58-3.60 (m, 2H), 2.86 (q, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 159.7, 144.6, 141.2, 139.7, 133.3, 130.8, 127.5, 117.6, 52.5, 48.7, 26.7, 13.3, 11.7; HRMS calcd for $C_{16}H_{17}ClN_4$ $[M+H]^+$ 301.1214, found 301.1217.

6,7-Dihydro-9-phenyl-3-(thiophen-2-yl)-5H-pyridazino[4,3-e][1,4]diazepine (3.46e)

White solid, 79% yield; Data for **3.46e**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.73-7.74 (m, 1H), 7.69-7.70 (m, 1H), 7.61-7.62 (m, 1H), 7.34-7.44 (m, 5H), 7.25 (s, 1H), 7.21-7.23 (m, 1H), 4.07-4.08 (m, 2H), 3.56-3.58 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.1, 151.2, 146.7, 141.8, 141.0, 140.5, 129.5, 129.1, 128.7, 128.4, 127.5, 126.1, 106.2, 52.3, 48.3; HRMS calcd for $C_{17}H_{14}N_4S$ $[M+H]^+$ 307.1012, found 307.1016.

6,7-Dihydro-3-phenyl-9-phenyl-5H-pyridazino[4,3-e][1,4]diazepine (3.46f)

Yellow solid, 77% yield; Data for **3.46f**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (m, 2H), 7.60-7.61 (m, 1H), 7.35-7.56 (m, 9H), 7.31 (s, 1H), 4.09-4.10 (m, 2H), 3.58-3.60 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.3, 155.3, 146.8, 141.9, 140.9, 136.3, 129.8, 129.1, 129.0, 128.7, 127.5, 126.7, 108.5, 52.4, 48.3; HRMS calcd for $C_{19}H_{16}N_4$ $[M+H]^+$ 301.1448, found 301.1446.

3-Cyclohexyl-6,7-dihydro-9-phenyl-5H-pyridazino[4,3-e][1,4]diazepine (3.46g)

White solid, 79% yield; Data for **3.46g**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.34-7.39 (m, 6H), 6.67 (s, 1H), 4.01-4.03 (m, 2H), 3.51-3.52 (m, 2H), 2.68-2.72 (m, 1H), 1.70-1.90 (m, 5H), 1.24-1.50 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.4, 164.3, 146.7, 142.0, 140.6, 129.1, 128.6, 127.4, 108.9, 52.3, 48.3, 43.3, 32.2, 26.1, 25.8; HRMS calcd for $C_{19}H_2N_4$ $[M+H]^+$ 307.1917, found 307.1909.

6,7-Dihydro-3-isopropyl-9-phenyl-5H-pyridazino[4,3-e][1,4]diazepine (3.46h)

White solid, 78% yield; Data for **3.46h**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.34-7.39 (m, 6H), 6.70 (s, 1H), 4.01-4.02 (m, 2H), 3.52-3.53 (m, 2H), 3.02-3.07 (m, 1H), 1.25 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.4, 165.2, 146.7, 142.0, 140.6, 129.1, 128.6, 127.4, 108.5, 52.3, 48.3, 33.5, 22.2; HRMS calcd for $C_{16}H_{18}N_4$ $[M+H]^+$ 267.1604, found 267.1602.

Method D: preparation of isoxazolo[4,5-c]pyridazine analogues 3.47:

A solution of **3.42** (1 mmol) in 5 mL of $POCl_3$ was heated at 95 °C for 4 hours, after which the reaction mixture was cooled down to room temperature. $POCl_3$ was evaporated and the residue was dissolved in EtOAc and washed 2 times with 20 mL of saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford the chlorinated pyridazine used directly without further purification for the next step.

To a solution of chlorinated pyridazine in 5 mL of EtOH and 1 mL of pyridine was added hydroxylamine hydrochloride (208 mg, 3 mmol) and refluxed for 16 hours. After conversion of the ketone into the corresponding oxime (monitored by TLC), the reaction mixture was allowed to cool to room temperature. CH_2Cl_2 was added and the organic layer was then washed 2 times with 10 mL of saturated aqueous $NaHCO_3$, and 1 time with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, to the residue obtained dissolved in THF was slowly added NaH 60% (52 mg, 1.3 mmol) under argon at room temperature. The reaction mixture was heated at 40 °C for 2 hours, after completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. CH_2Cl_2 was added and the organic layer was then washed 2 times with 10 mL of saturated aqueous $NaHCO_3$, and 1 time with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:4) to obtain the desired isoxazolo[4,5-c]pyridazine **3.47**.

3-(4-Chlorophenyl)-7-methyl-6-ethyl-isoxazolo[4,5-c]pyridazine (3.47a)

Beige solid, 61% yield; Data for **3.47a**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.55 (d, *J* = 8.7 Hz, 2H), 7.78 (d, *J* = 8.7 Hz, 2H), 3.20 (q, *J* = 7.5 Hz, 2H), 2.60 (s, 3H), 1.37 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.3, 156.2, 154.4, 144.6, 136.8, 129.9, 129.7, 125.2, 117.3, 25.8, 13.3, 10.4; HRMS calcd for $C_{14}H_{12}ClN_3O$ $[M+H]^+$ 274.0742, found 274.0743.

3-Phenyl-6-(thiophen-2-yl)-isoxazolo[4,5-c]pyridazine (3.47b)

Orange solid, 46% yield; Data for **3.47b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.52-8.54 (m, 2H), 8.15 (d, *J* = 3.7 Hz, 1H), 7.88 (d, *J* = 5.0 Hz, 1H), 7.71-7.72 (m, 3H), 7.32 (t, *J* = 5.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 156.7, 155.1, 153.0, 145.9, 139.6, 132.1, 131.4, 129.5, 128.9, 128.7, 128.3, 125.9, 101.8; HRMS calcd for $C_{15}H_9N_3OS$ $[M+H]^+$ 280.0539, found 280.0543.

Sodium benzene sulfinate (3.49)

A solution of sodium sulfite (627 mg, 4.98 mmol) and sodium bicarbonate (418 mg, 4.98 mmol) in 20 mL of water was stirred vigorously with benzenesulfonyl chloride (301 μL, 2.36 mmol) at 75 °C for 5 h. After cooling to ambient temperature, the reaction mixture was directly lyophilized. The resulting white solid was stirred with 20 mL of methanol for 10 minutes and the insoluble inorganic salts removed by filtration. The filtrate was concentrated in vacuo to approximately 5 mL and an equal volume of diethyl ether was added. The precipitated solid containing residual inorganic salts was filtered and set aside. The

remaining filtrate was diluted with excess diethyl ether, filtered and the filtered solid dried in vacuo to give sodium 3-chloro-benzenesulfinate in 73% as a white solid used directly without further purification.

1-Phenylsulfonyl-2-propanone (3.51)

To a solution of chloroacetone (80 μL, 1.0 mmol) in 10 mL of DMF was added at room temperature **3.49** (246 mg, 1.5 mmol). After stirring 24 h at room temperature, the reaction mixture was diluted with 20 mL of Et_2O and 20 mL of water. The aqueous phase was extracted with 3x15 mL Et_2O before washing with 10 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the sulfonyl acetone **3.51** in 52% yield as pale yellow solid. Data for **3.51**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80-7.92 (m, 2H), 7.58-7.72 (m, 3H), 4.15 (s, 2H), 2.24 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 141.2, 135.7, 131.0, 128.7, 68.9, 33.2.

General procedure for the preparation of sulfonyl derivatives 3.52:

To a solution of lithium diisopropylamine 2M (1.1 mL, 2.2 mmol) in 40 mL of anhydrous THF at -78 °C was added dropwise a solution of 1-phenylsulfonyl-2-propanone **3.51** (198 mg, 1.0 mmol) in 10 mL of THF. After 4 h at -78 °C, an aldehyde (1.1 mmol) was added to the resulting orange heterogenous solution and the reaction mixture was allowed to warm to room temperature overnight. Hydrolysis was achieved at 0 °C with 30 mL of saturated aqueous NH_4Cl, fllowed by an addition of AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the aldol derivatives **3.52**.

4-Hydroxy-1-(phenylsulfonyl)-hexan-2-one (3.52a)

Yellow oil, 70% yield; Data for **3.52a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (d, *J* = 7.1 Hz, 2H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.66 (t, *J* = 7.1 Hz, 2H), 4.71 (d, *J* = 3.6 Hz, 2H), 4.67 (d, *J* = 5.6 Hz, 1H), 3.72-3.78 (m, 1H), 2.58 (d, *J* = 6.3 Hz, 2H), 1.25-1.36 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.4, 144.8, 140.3, 133.7, 129.1, 66.9, 64.7, 53.1, 28.7, 12.2.

4-Hydroxy-4-(4-methoxyphenyl)-1-(phenylsulfonyl)-butan-2-one (3.52b)

Yellow oil, 63% yield; Data for **3.52b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, *J* = 7.3 Hz, 2H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.64 (t, *J* = 7.3 Hz, 2H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.34 (d, *J* = 4.6 Hz, 1H), 4.88-4.94 (m, 1H), 4.73 (d, *J* = 4.5 Hz, 2H), 3.73 (s, 3H), 2.74-2.93 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 197.4, 145.7, 139.5, 134.0, 130.8, 129.3, 128.0, 127.0, 123.8, 67.7, 65.5, 55.1, 53.2.

4-Hydroxy-1-(phenylsulfonyl)-4-(thiophen-2-yl)-butan-2-one (3.52c)

Yellow oil, 50% yield; Data for **3.52c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (d, *J* = 7.2 Hz, 2H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.2 Hz, 2H), 7.38-7.40 (m, 1H), 6.93-6.96 (m, 1H), 6.89-6.90 (m, 1H),

5.84 (d, *J* = 5.1 Hz, 1H), 5.18-5.24 (m, 1H), 4.76 (d, *J* = 2.3 Hz, 2H), 2.90-3.08 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.9, 149.3, 139.4, 134.1, 129.4, 128.0, 126.7, 124.5, 123.1, 65.5, 64.1, 53.2.

General procedure for the preparation of pyridazine derivatives 3.54:

To a solution of compound **3.52** (1.0 mmol) in 30 mL of acetonitrile under argon at 0°C was added successively triethylamine (181 μL, 1.3 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (240 mg, 1.0 mmo). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 50 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the desired diazo derivative **3.53**.

To a solution of **3.53** (1 mmol) in 10 mL of acetonitrile under argon was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was allowed to warm to room temperature and then filtered. The filtrate was concentrated and used directly without further purification for the next step.

To the previously prepared crude in 15 mL of *i*-Pr_2O was added P(*n*-Bu)$_3$ (250 μL, 1.0 mmol). The reaction mixture was stirred under argon at room temperature for 30 minutes, after which a purification by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 9:1) due to no formation of precipitate was performed to obtain the desired sulfonyl pyridazine analogues **3.54**.

6-Ethyl-3-(phenylsulfonyl)-pyridazin-4-ol (3.54a)

Beige solid, 40% yield; Data for **3.54a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, *J* = 7.2 Hz, 2H), 7.74 (t, *J* = 7.4 Hz, 1H), 7.63 (t, *J* = 7.3 Hz, 2H), 6.47 (s, 1H), 2.57 (q, *J* = 7.6 Hz, 2H), 1.17 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.9, 157.7, 151.8, 138.6, 134.3, 129.1, 128.8, 119.3, 23.8, 12.3.

6-(4-Methoxyphenyl)-3-(phenylsulfonyl)pyridazin-4-ol (3.54b)

Orange solid, 29% yield; Data for **3.54b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.02 (d, *J* = 7.4 Hz, 2H), 7.73-7.76 (m, 3H), 7.65 (t, *J* = 7.7 Hz, 2H), 7.10 (d, *J* = 8.9 Hz, 2H), 6.86 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.9, 151.9, 138.8, 134.3, 129.2, 129.1, 129.0, 122.1, 118.0, 114.8, 55.7.

3-(Phenylsulfonyl)-6-(thiophen-2-yl)pyridazin-4-ol (3.54c)

Pale yellow solid, 20% yield; Data for **3.54c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (d, *J* = 7.3 Hz, 2H), 7.93 (d, *J* = 4.4 Hz, 1H), 7.85 (d, *J* = 3.7 Hz, 1H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.66 (t, *J* = 7.2 Hz, 2H), 7.27-7.30 (m, 1H), 6.89 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.4, 151.8, 138.8, 134.3, 131.8, 129.7, 129.3, 129.1, 129.0.

Methyl 2-diazo-3-oxopentanoate (3.25b)

To a solution of methyl 3-oxopentanoate (5 g, 38.42 mmol) in 30 mL of acetonitrile under argon at 0°C was added successively triethylamine (6.9 mL, 49.95 mmol) and *p*-acetamido benzene sulfonyl azide (*p*-

ABSA) (9.2 g, 38.42 mmol). The mixture was stirred for 2 hours and was allowed to warm to room temperature and was diluted with 100 mL of Et_2O/*n*-Hexane (1:1) and then filtered. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:2) to afford the desired compound methyl 2-diazo-3-oxopentanoate in 85% yield as yellow oil. Data for **3.25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.77 (s, 3H), 2.78 (q, *J* = 7.3 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 192.5, 161.5, 52.3, 33.0, 8.2. Diazo carbon was not detected in ^{13}C NMR.

Methyl 2-diazo-5-(2-fluorophenyl)-5-hydroxy-3-oxopentanoate (3.22h)

Yellow oil, 69% yield; Data for **3.22h**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50-7.55 (m, 1H), 7.27-7.30 (m, 1H), 7.10-7.23 (m, 2H), 5.51 (d, *J* = 5.0 Hz, 1H), 5.33-5.39 (m, 1H), 3.77 (s, 3H), 3.34-3.43 (m, 1H), 2.93 (dd, *J* = 15.8 Hz, *J* = 4.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.3, 161.4, 160.7, 157.4, 131.8, 129.0, 127.9, 124.5, 115.2, 75.9, 62.6, 52.4, 47.6; HRMS calcd for $C_{12}H_{13}N_2O_2$ $[M+H]^+$ 243.1128, found 243.1130.

Methyl 2-diazo-5-hydroxy-4-methyl-3-oxoheptanoate (3.22i)

Yellow oil, 88% yield; Inseparable diastereomeric mixture; Data for **3.22i** (major isomer): ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.54 (d, *J* = 6.2 Hz, 1H), 3.77 (s, 3H), 3.48-3.60 (m, 2H), 1.25-1.42 (m, 2H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.86 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.6, 161.4, 72.4, 52.3, 47.1, 27.9, 11.6, 10.6; HRMS calcd for $C_{14}H_{14}N_2O_2$ $[M+Na]^+$ 243.1128, found 243.1130. Diazo carbon was not detected in ^{13}C NMR.

Methyl 5-cyclohexyl-2-diazo-5-hydroxy-4-methyl-3-oxopentanoate (3.22k)

Yellow oil, 63% yield; Inseparable diastereomeric mixture; Data for **3.22k** (major isomer): ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.41 (d, *J* = 6.8 Hz, 1H), 3.78 (s, 3H), 3.60-3.64 (m, 1H), 3.42-3.48 (m, 1H), 1.51-1.87 (m, 6H), 1.08-1.28 (m, 5H), 0.98 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.6, 161.3, 75.9, 74.5, 52.3, 44.7, 41.0, 29.3, 28.4, 26.2, 26.0, 25.9, 10.6; HRMS calcd for $C_{14}H_{14}N_2O_2$ $[M+Na]^+$ 243.1128, found 243.1130.

Methyl 2-diazo-5-hydroxy-5-(4-methoxyphenyl)-4-methyl-3-oxopentanoate (3.22l)

Yellow oil, 53% yield; Data for **3.22l**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.21-5.23 (m, 1H), 4.79-4.80 (m, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 3.30-3.31 (m, 1H), 0.97 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 193.5, 161.4, 158.2, 135.9, 127.3, 113.3, 72.3, 55.1, 52.4, 49.3, 11.0; HRMS calcd for $C_{14}H_{14}N_2O_2$ $[M+H]^+$ 243.1128, found 243.1130. Diazo carbon was not detected in ^{13}C NMR.

Methyl 2-diazo-5-(2-fluorophenyl)-5-hydroxy-4-methyl-3-oxopentanoate (3.22m)

Yellow oil, 51% yield; Data for **3.22m**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47 (m, 1H), 7.31-7.38 (m, 1H), 7.22-7.30 (m, 1H), 7.12-7.18 (m, 1H), 5.52 (t, *J* = 4.4 Hz, 1H), 5.04-5.09 (m, 1H), 3.90-3.95 (m, 1H), 3.80 (s, 3H), 0.76 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.3, 161.3, 158.0, 130.5, 129.3, 128.8, 124.7, 115.1, 75.8, 67.9, 52.4, 47.8, 13.2; HRMS calcd for $C_{14}H_{14}N_2O_2$ $[M+H]^+$ 243.1128, found 243.1130.

General procedure for the catalytic Diaza-Wittig reaction:

To a solution of **3.22** (1 mmol) in 10 mL of acetonitrile under argon at 0°C was added IBX (1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide) (364 mg, 1.3 mmol). The mixture was refluxed for 2 hours and was allowed to warm to room temperature and then filtered. The filtrate was concentrated and used directly without further purification for the next step.

To the previously prepared crude in 5 mL of toluene under argon was added **3.57** (19 mg, 0.1 mmol) followed by diphenylsilane (205 µL, 1.1 mmol). The reaction mixture was heated at 100°C for 16 hours. After completion of the reaction monitored by TLC, the reaction mixture was allowed to cool to room temperature and a precipitate was formed. The precipitate was filtered and washed with diisopropyl ether to afford the pyridazine **3.24**.

Methyl 6-(2-fluorophenyl)-4-hydroxy-pyridazine-3-carboxylate (3.24h)

Pale yellow solid, 82% yield; Data for **3.24h**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.77 (br. s, 1H), 7.65-7.73 (m, 2H), 7.39-7.49 (m, 2H), 6.74 (s, 1H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.0, 160.7, 157.4, 133.5, 133.4, 131.0, 130.9, 125.4, 125.3, 118.9, 116.5, 52.6; HRMS calcd for $C_{12}H_9FN_2O_3$ $[M+H]^+$ 249.0670, found 249.0675.

Methyl 6-ethyl-4-hydroxy-5-methyl-pyridazine-3-carboxylate (3.24i)

White solid, 81% yield; Data for **3.24i**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.27 (br. s, 1H), 3.80 (s, 3H), 2.64 (q, *J* = 7.5 Hz, 2H), 1.93 (s, 3H), 1.17 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.4, 164.6, 152.8, 145.7, 125.9, 52.3, 22.5, 12.8, 9.3; HRMS calcd for $C_9H_{12}N_2O_3$ $[M+H]^+$ 197.0921, found 197.0927.

Methyl 4-hydroxy-5-methyl-6-phenyl-pyridazine-3-carboxylate (3.24j)

White solid, 63% yield; Data for **3.24j**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.54 (br. s, 1H), 7.57-7.58 (m, 5H), 3.84 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 164.4, 149.8, 145.5, 130.9, 130.4, 129.2, 128.9, 126.8, 52.4, 11.3; HRMS calcd for $C_{13}H_{12}N_2O_3$ $[M+H]^+$ 245.0921, found 245.0921.

Methyl 6-cyclohexyl-4-hydroxy-5-methyl-pyridazine-3-carboxylate (3.24k)

White solid, 75% yield; Data for **3.24k**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.97 (br. s, 1H), 3.80 (s, 3H), 2.82-2.90 (m, 1H), 1.97 (s, 3H), 1.56-1.83 (m, 7H), 1.20-1.44 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6)

δ 167.4, 164.5, 154.7, 145.3, 125.4, 52.3, 38.4, 29.7, 25.9, 25.2, 9.3; HRMS calcd for $C_{13}H_{18}N_2O_3$ $[M+H]^+$ 251.1390, found 251.1391.

Methyl 4-hydroxy-6-(4-methoxyphenyl)-5-methyl-pyridazine-3-carboxylate (3.24l)

White solid, 70% yield; Data for **3.24l**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.45 (br. s, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.8, 164.4, 160.7, 149.6, 145.3, 130.7, 126.6, 122.9, 114.2, 55.5, 52.3, 11.3; HRMS calcd for $C_{14}H_{14}N_2O_4$ $[M+H]^+$ 275.1026, found 275.1028.

Methyl 6-(2-fluorophenyl)-4-hydroxy-5-methyl-pyridazine-3-carboxylate (3.24m)

White solid, 78% yield; Data for **3.24m**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.70 (br. s, 1H), 7.60-7.71 (m, 2H), 7.41-7.50 (m, 2H), 3.85 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.5, 164.3, 160.6, 157.3, 145.7, 144.5, 133.2, 131.5, 128.6, 125.2, 118.3, 116.2, 52.5, 11.2; HRMS calcd for $C_{13}H_{11}FN_2O_3$ $[M+H]^+$ 263.0826, found 263.0826.

(2-azido-acetamido)-5-chloro-benzophenone (3.64)

To a solution of 2-amino-5-chloro-benzophenone **3.62** (500 mg, 2.16 mmol) and azido acetic acid **3.63** (262 mg, 2.59 mmol) in 5 mL of CH_2Cl_2 under argon at room temperature was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (497 mg, 2.59 mmol). After stirring at room temperature overnight, 10 mL of saturated aqueous NH_4Cl was added. The organic layer was separated and then washed with 20 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted with 20 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) to afford the desired amide **3.64** in 88% yield as Pale yellow solid; Data for **3.64**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (br. s, 1H), 7.64-7.70 (m, 5H), 7.53 (t, *J* = 7.7 Hz, 2H), 7.45 (s, 1H), 3.79 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 193.9, 166.5, 136.6, 134.6, 133.2, 132.5, 131.8, 129.7, 129.6, 128.9, 128.5, 125.9, 51.2; HRMS calcd. for $C_{15}H_{11}ClN_4O_2$ $[M+H]^+$ 315.0643, found 315.0641.

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (3.65)

To a solution of **3.64** (598 mg, 1.90 mmol) in 5 mL of toluene under argon was added **3.57** (37 mg, 0.19 mmol) followed by diphenylsilane (389 μL, 2.09 mmol). The reaction mixture was heated at 100°C for 16 hours. After completion of the reaction monitored by TLC, 10 mL of saturated aqueous NH_4Cl was added. The organic layer was separated and then washed with 10 mL of saturated aqueous $NaHCO_3$. The aqueous layers were extracted with 10 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/MeOH = 9:1) to obtain **3.65** in 77% yield) as white solid; Data for **3.65**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.65 (br. s, 1H), 7.65 (dd, *J* = 8.8 Hz, *J* = 2.3 Hz, 1H), 7.44-7.64 (m, 5H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 2.2 Hz, 1H), 4.16 (br. s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.3, 168.5, 149.7,

138.7, 131.6, 130.5, 129.8, 129.3, 128.5, 127.8, 126.5, 123.2, 57.1; HRMS calcd. for $C_{15}H_{11}ClN_2O$ $[M+H]^+$ 271.0633, found 271.0637.

Diazepam (3.66)

To a solution of **3.65** (100 mg, 0.37 mmol) in 5 mL of THF under argon was added NaH 60% (18 mg, 0.44 mmol), and after 30 minutes iodomethane (24 μL, 0.39 mmol). The reaction mixture was stirred at room temperature overnight. After completion of the reaction monitored by TLC, 10 mL of saturated aqueous $NaHCO_3$ was added. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc/*n*-Hexane = 8:2) to afford the Diazepam **3.66** in 52% yield as white solid; Data for **3.66**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.0 Hz, 2H), 7.47-7.52 (m, 2H), 7.41 (t, *J* = 6.6 Hz, 2H), 7.30 (d, *J* = 5.8 Hz, 1H), 7.29 (s, 1H), 4.84 (d, *J* = 10.8 Hz, 2H), 3.77 (d, *J* = 10.8 Hz, 2H), 3.39 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 168.9, 142.6, 138.2, 131.4, 130.7, 129.9, 129.5, 128.4, 122.5, 56.9, 34.9; HRMS calcd. for $C_{16}H_{13}ClN_2O$ $[M+H]^+$ 285.0789, found 285.0783.

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Chapter IV. Synthesis and biological evaluation of triazolo[4,3-a]pyridine, triazolo[4,5-b]pyridine and triazolo[1,5-a]pyridine libraries

1. Introduction

The last thirty years have seen an increasing interest in the use of various triazolopyridines in pharmaceutical industry.[1] Five isomers can be distinguished and have been studied and evaluated by several groups.

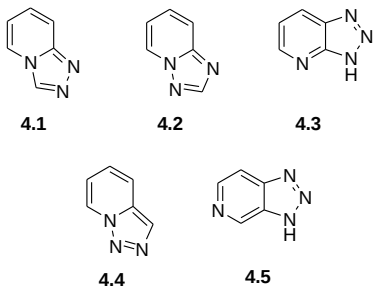


Figure 1. Triazolopyridine isomers.

Among these isomers of triazolopyridine, it is possible to divide them in two categories. Three of them possess a bridgehead nitrogen in the fusion (**4.1**, **4.2**, **4.4**) in contrast to the two others isomers which contain a free amine on the triazole part (**4.3**, **4.5**) and could be used as point of substitution. One project of the PhD was dedicated to the construction of libraries based on the scaffold **4.1**, **4.2**, **4.3**.

The resemblance between purine and the triazolo[4,5-b]pyridine **4.3** have led to glycosyl derivatives such as **4.6** tested against HIV-l.[2] This class of fused ring has been associated with a wide range of different biological applications, in kinase inhibition, several analogues displayed activity towards various targets. Galapagos discovered the first selective JAK1 inhibitor[3] based on triazolo[1,5-a]pyridine **4.7**. Almirall Research[4] has designed a kinase inhibitor by combining a tolyl amide motif and a triazolo[4,3-a]pyridine to obtain a selective and potent p38 MAP inhibitor **4.8**.

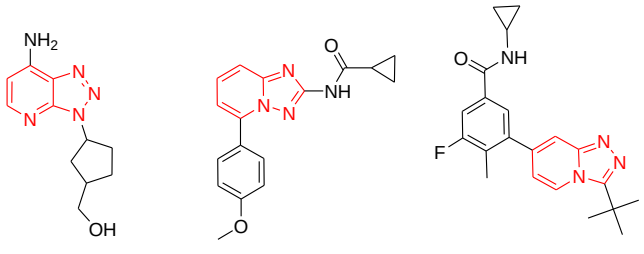
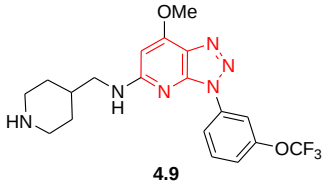


Figure 2. Biologically active triazolopyridine derivatives.

In 2012, Pastor *et al.*[5] published a study concerning a hit to lead evaluation of novel Pim kinase inhibitors. Their research led to the discovery of a triazolo[4,5-b]pyridine with a pattern of substitution similar to the scaffold investigated during the PhD research. **4.9** showed potency and selectivity towards Pim-1 kinase (IC_{50} = 1 nM), but further data proved risk of cardiotoxicity.

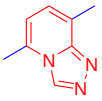


4.9

Figure 3. Pim-1 kinase inhitor.

2. Results and discussion

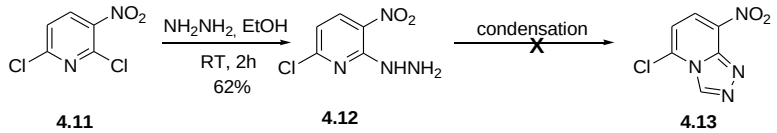
The first scaffold investigated was the triazolo[4,3-a]pyridine **4.1**, for the project it was decided to design a scaffold with two points of substitution. After a review of the existing literature, the positions 5 and 8 have been chosen for the development of library based on the structure **4.10**.



4.10

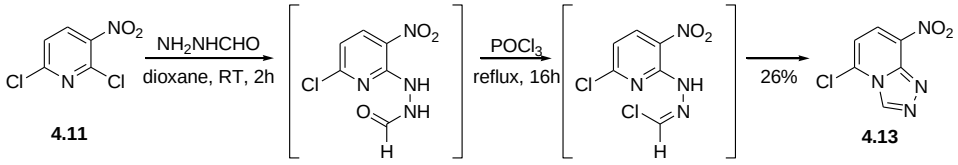
Figure 4. Scaffold investigated.

A large number of methods of synthesis exist to obtain the triazolo[4,3-a]pyridine such as palladium cross-coupling with hydrazides followed by condensation[6] but in our case a simple nucleophilic substitution prior to the final condensation[7] was studied. The first step was the hydrazinolysis of 2,6-dichloro-3-nitro-pyridine **4.11** in ethanol at room temperature for 1 hour to afford the hydrazino derivative **4.12**. The condensation has been attempted with (trialkyl)orthoformate reagent but did not deliver the desired product **4.13**, the condensation reagent has been used also as solvent or with catalytic amount of acid, no improvements have been observed, the cyclization has been tried with a more reactive condensation reagent, the Vilsmeier-Hack reagent generated by doing the reaction in DMF with a stoechiometric amount of phosphorus oxychloride, unfortunately the same results have been obtained.



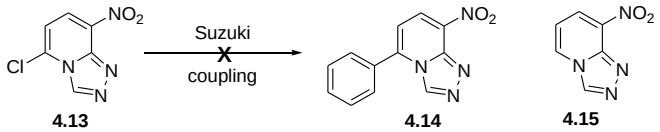
Scheme 1. Attempts of synthesis of 5-chloro-8-nitro-triazolo[4,3-a]pyridine.

In order to complete the pathway for the synthesis of the scaffold, another reagent has been investigated, the formic acid hydrazide could allow, after a nucleophilic substitution, the formation of a reactive intermediate in phosphorus oxychloride towards the attack of the nitrogen of the pyridine leading to the triazolo[4,3-a]pyridine **4.13**. This conversion is a one-pot reaction, **4.13** was obtained in 26% yield.



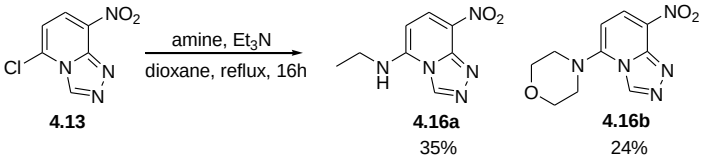
Scheme 2. Synthesis of 5-chloro-8-nitro-triazolo[4,3-a]pyridine with formic acid hydrazide.

The synthesis of **4.13** achieved, the substitution of the chloride was the next step and due to the presence of the nitro group in the para position to increase the reactivity, it was expected that a strategy based on palladium cross-coupling or nucleophilic substitution could be straightforward. Our first attempt was to perform a Suzuki cross-coupling between **4.13** and phenyl boronic acid, various conditions have been used but the starting material was always recovered and no desired product 4.14 or unchlorinated by-product **4.15** were observed.



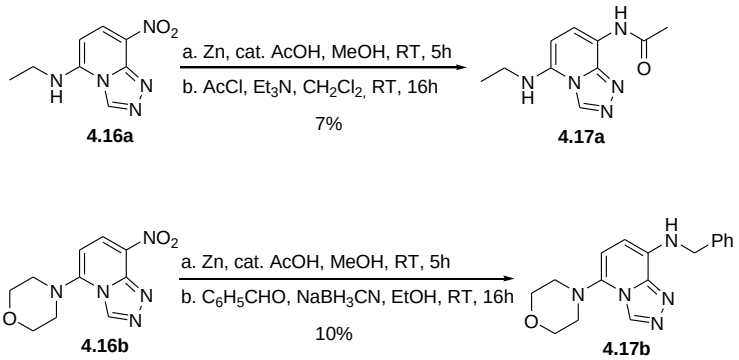
Scheme 3. Attempts of Suzuki reaction.

Instead of a palladium cross-coupling, we evaluated the substrate towards nucleophilic substitution with simple amines such as ethylamine or morpoline. After 16 hours of reaction in refluxing dioxane, substituted triazolo[4,3-a]pyridine **4.16** were obtained in low yields.



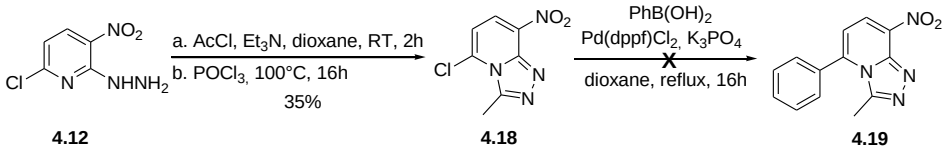
Scheme 4. Nucleophilic substitution.

The derivative **4.16** has been then reduced with zinc to the corresponding amine, used directly without further purification for the next step. **4.16a** has been acyclated by acetyl chloride after reduction and the amine derivative obtained from **4.16b** was subjected to a reductive amination with benzaldehyde. The overall yield of this strategy is very low, and the first step leading to the intermediate **4.13** was not correctly reproducible, for all these reasons **4.17a** and **4.17b** are the only examples synthesized with this pattern of substitution for the triazolo[4,3-a]pyridine. Our interest was then directed towards the same scaffold with an additional substitution such as methyl at the position 3, for this purpose **4.12** was acylated with acetyl chloride at room temperature for 2 hours, after which time, phosphorus oxychloride was added to perform the cyclization at 100°C for 16 hours, **4.18** was obtained in 35%.



Scheme 5. Synthesis of triazolo[4,3-a]pyridine derivatives **4.17**.

4.18 was subjected to Suzuki cross-coupling with phenyl boronic acid but led to identical results than **4.13**. The non reactivity of the scaffold towards previously discussed reaction and the low yields obtained forced us to modify the pattern of substitution. In fact, the nitro group has been kept at the position 8 but instead of to attempt palladium coupling at the position 5 to attach aryl group, it was envisioned to introduce substitution at the position 3 of the triazole part.



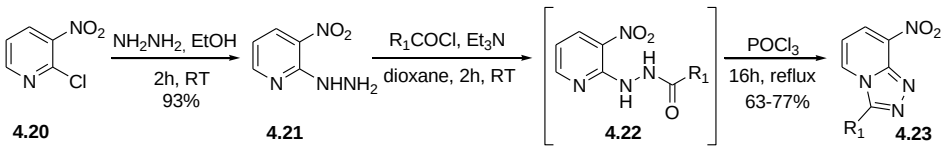
Scheme 6. Attempt of Suzuki coupling with 3-methyl-triazolo[4,3-a]pyridine derivatives **4.18**.

It was decided to remove the chloride at the position 5 and to have a scaffold with a point of substitution on the pyridine and the second one on the triazole part. The

introduction of variety at the position 3 was possible by using an appropriate acid chloride during the cyclization to afford 8-nitro-3-substituted-triazolo[4,3-a]pyridine.

A similar method than the synthesis of **4.18** could be used the 2-chloro-3-nitro-pyridine **4.20** has been selected as starting material, in fact this starting material had two advantages for the procedure envisioned, the first one was a functional group (nitro group) allowing the possible synthesis of a library, then the second advantage was the chloride easily replaceable by nucleophilic substitution due to the electron withdrawing effect of the nitro group at its ortho position.

The first step was the hydrazinolysis of **4.20** with hydrazine hydrate in ethanol at room temperature for 3 hours to afford a yellow solid **4.21** in 93% yield, then the formation of the scaffold was performed in a two steps one-pot reaction. An acylation of the hydrazino derivative **4.21** was first done with an acid chloride to obtain an intermediate **4.22** which after complete disappearance of **4.21** (monitored by TLC) was treated with a dehydrating reagent allowing the cyclization to lead to the triazolo[4,3-a]pyridine **4.23**. Different dehydrating reagents have been screened, but the phosphorus oxychloride gave the best results for the cyclization.

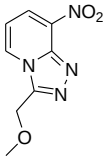
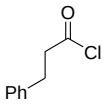
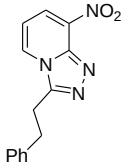
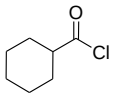
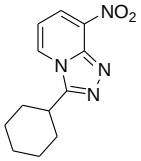
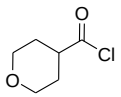
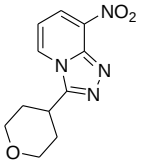
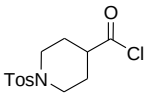
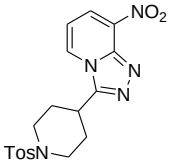
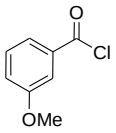
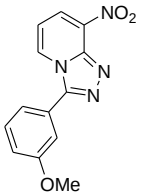
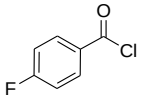
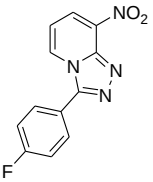
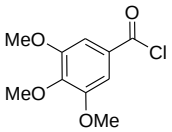
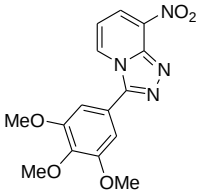
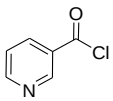
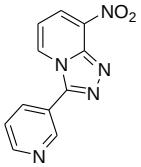


4.20 **4.21** **4.22** **4.23**

Scheme 7. Synthesis of triazolo[4,3-a]pyridine derivatives **4.23**.

This method led to the synthesis of nine triazolo[4,3-a]pyridine derivatives **4.23** containing various substitutions at the position 3, the introduction of (hetero)aryl, cycloalkyl or aliphatic groups was possible in good yields. This pathway showed that a library based on the triazolo[4,3-a]pyridine substituted at the position 3 was simpler to build in contrast to the initially investigated scaffold with a hydrogen on the triazole part, one possible explanation is tendency of the triazole part to reopen as it will be discussed later.

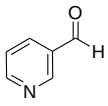
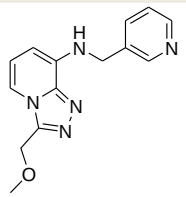
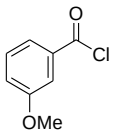
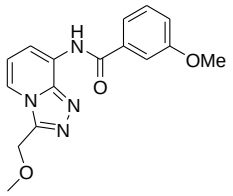
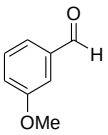
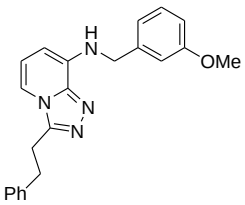
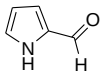
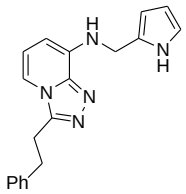
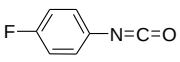
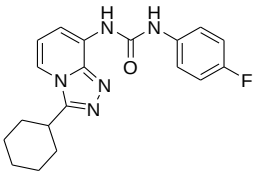
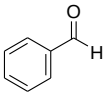
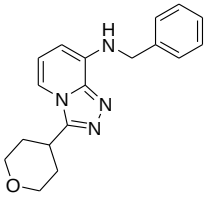
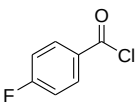
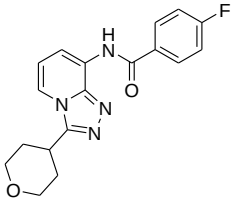
Table 1. Synthesis of 8-nitro-triazolo[4,3-a]pyridine derivatives **4.23**.[a]

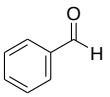
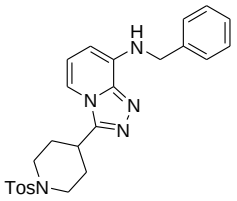
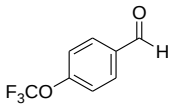
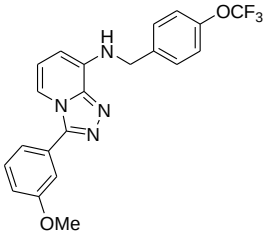
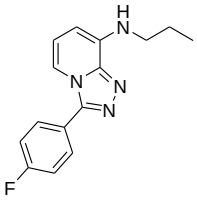
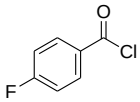
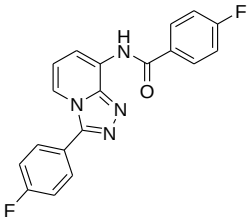
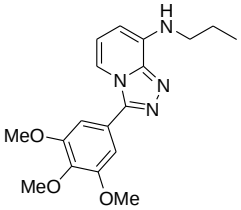
entry	starting material	acid chloride	triazolo[4,3-a]pyridine		yield (%)[b]
1	**4.21**			**4.23a**	77
2	**4.21**			**4.23b**	65
3	**4.21**			**4.23c**	71
4	**4.21**			**4.23d**	67
5	**4.21**			**4.23[e]**	63
6	**4.21**			**4.23f**	75
7	**4.21**			**4.23g**	70
8	**4.21**			**4.23h**	63
9	**4.21**			**4.23i**	69

[a]All reactions were performed using 1 mmol of **4.21**, 1.05 mmol of acid chloride and 181 μL of triethylamine in dioxane at room temperature for 2 hours, after which 135 μL of $POCl_3$ was added, the reaction mixture was refluxed for 16 hours. [b]Isolated yield.

The nitro derivative **4.23** in hands, the synthesis of a library could start. The corresponding amine was obtained by reduction of **4.23** with zinc and a catalytic amount of acetic acid and then subjected to a series of reaction such as acylation or reductive amination to afford a collection of analogues **4.24**.

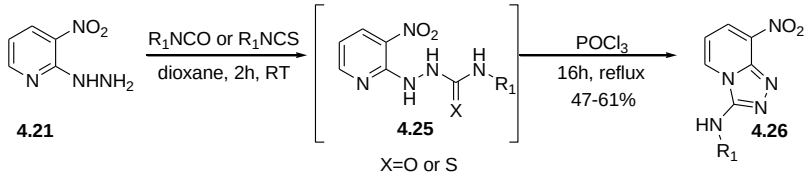
Table 2. Synthesis of triazolo[4,3-a]pyridine derivatives **4.24**.[a]

entry	starting material	reagent	triazolo[4,3-a]pyridine		yield (%)[b]
1	**4.23a**			**4.24a**	29
2	**4.23a**			**4.24b**	31
3	**4.23b**			**4.24c**	23
4	**4.23b**			**4.24d**	14
5	**4.23c**			**4.24[e]**	11
6	**4.23d**			**4.24f**	19
7	**4.23d**			**4.24g**	33

8	**4.23e**			**4.24h**	19
9	**4.23f**			**4.24i**	22
10	**4.23g**			**4.24j**	28
11	**4.23g**			**4.24k**	25
12	**4.23h**			**4.24l**	27

[a]See the experimental section for details. [b]Isolated yield.

Based on this strategy, the synthesis of triazolo[4,3-a]pyridine with nitrogen linker at the position 3 has been done by replacing the acid chloride by an isocyanate or isothiocyanate reagents for the cyclization step. Intermediate **4.21** was treated with these reagents to generate a (thio)semicarbazide derivative **4.25** which after treatment with phosphorus oxychloride led to the desired triazolo[4,3-a]pyridine **4.26** with a substitution at the position 3 attached by a nitrogen, this modification allowed the formation of a hinge binder with the aromatic nitrogen at the position 2 and the new one added.

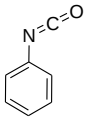
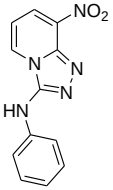
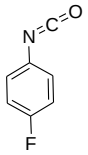
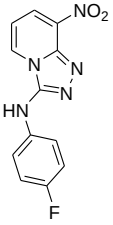
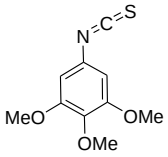
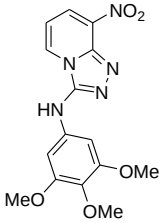
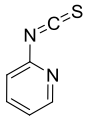
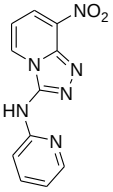


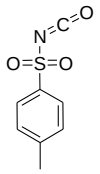
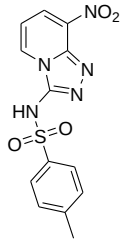
Scheme 8. Synthesis of 3-amino substituted triazolo[4,3-a]pyridine derivatives **4.26**.

R_1 was covering a five different decoration from aryl substituted or not to heteroaryl groups.

The amino substituted triazolo[4,3-a]pyridine **4.26** was obtained in moderate yields, most of the starting materials were isocyanate derivatives and proved to be better cyclization partner compared to the isothiocyanate analogue which gave the lower yield. It was possible to synthesize a scaffold with a sulfonamide linker instead of a simple amine by using a sulfonyl isocyanate reagent. The initial purpose of the derivative **4.26e** containing a sulfonamide was the possible cleavage of the p-toluene sulfonyl group with a solution of sodium hydroxide but due to the sensitivity of the triazolo[4,3-a]pyridine towards acidic or basic conditions, the removal of the protective group has been abandoned.

Table 3. Synthesis of 8-nitro-triazolo[4,3-a]pyridine derivatives **4.26**.[a]

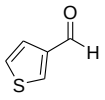
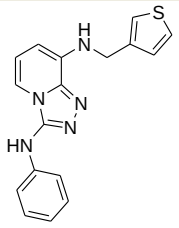
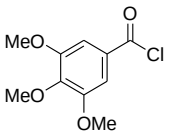
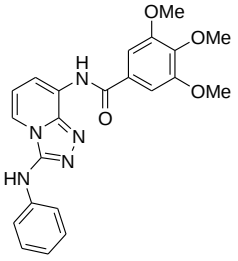
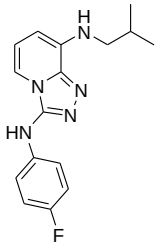
entry	starting material	reagent	triazolo[4,3-a]pyridine		yield (%)[b]
1	**4.21**			**4.26a**	58
2	**4.21**			**4.26b**	61
3	**4.21**			**4.26c**	49
4	**4.21**			**4.26d**	55

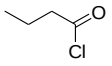
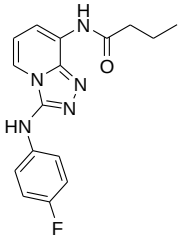
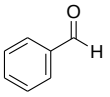
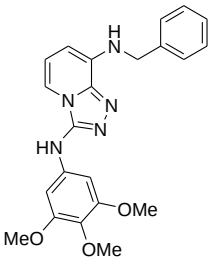
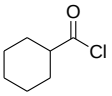
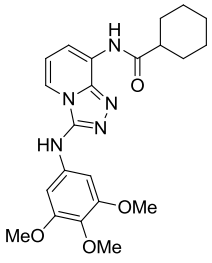
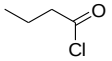
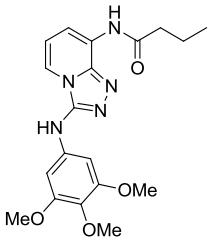
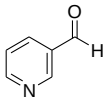
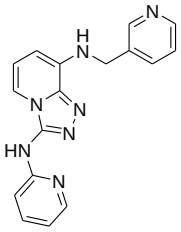
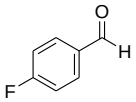
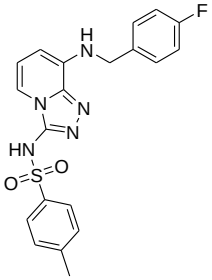
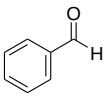
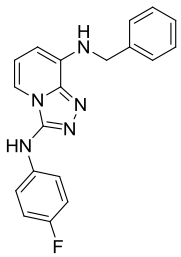
5	**4.21**			**4.26e**	47

[a]All reactions were performed using 1 mmol of **4.21** and 1.05 mmol of isocyanate or isothiocyanate in dioxane at room temperature for 2 hours, after which 135 μL of $POCl_3$ was added, the reaction mixture was refluxed for 16 hours. [b]Isolated yield.

The nitro group was then reduced like previously discussed to the corresponding amine and different reactions have been done to build a library of **4.27**. Due to the presence of two amines after the reduction of the nitro group, further modification could lead to a mixture, it was necessary to check the reactivity of the secondary amine attached at the position 3 of the scaffold. A review of the existing literature[8] removed the doubts concerning a possible competitive substitution, in fact the conditions used to react with the nitrogen linked to the triazole ring were harsher compared to the conditions used for the amine at the position 8.

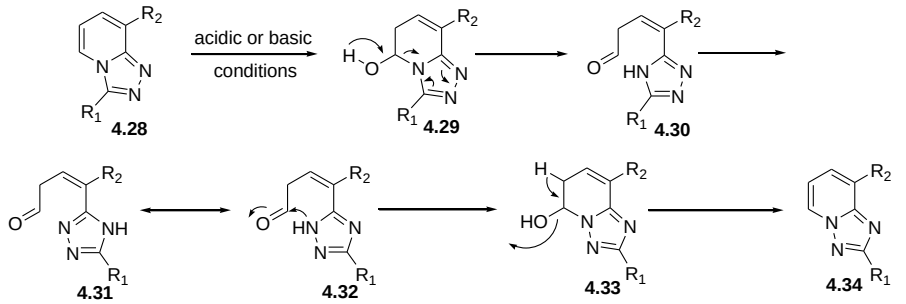
Table 4. Synthesis of triazolo[4,3-a]pyridine derivatives **4.27**.[a]

entry	starting material	reagent	triazolo[4,3-a]pyridine		yield (%)[b]
1	**4.26a**			**4.27a**	28
2	**4.26a**			**4.27b**	13
3	**4.26b**			**4.27c**	21

4	**4.26b**			**4.27d**	37
5	**4.26c**			**4.27[e]**	23
6	**4.26c**			**4.27f**	12
7	**4.26c**			**4.27g**	13
8	**4.26d**			**4.27h**	11
9	**4.26e**			**4.27i**	16
10	**4.26b**			**4.27j**	10

[a]See the experimental section for details. [b]Isolated yield.

During the quality control of the different derivatives **4.24** and **4.27** synthesized, it has been observed two distinct peaks in the LC/MS analysis with the same mass, after a review of the literature concerning this chemistry,[9] it has been found that a possible rearrangement could occur in acidic or basic pH to convert the triazolo[4,3-a]pyridine **4.1** to the triazolo[1,5-a]pyridine **4.2**, this transformation is known as the Dimroth rearrangement,[10] moreover it was described that the rearrangement was favored in the presence of a nitro group at the position 8 like in our case, in fact the electron withdrawing nitro group enhances the reactivity of the position 5 towards possible attack in acidic or basic conditions. In our particular case with a nitro group at the position 8 and no substitution at the position 5 of the triazolo[4,3-a]pyridine, the Dimroth rearrangement was observed frequently. A proposed mechanism of this rearrangement is depicted below.



Scheme 9. Proposed mechanism for the Dimroth rearrangement.

Derivative **4.28** generated an intermediate **4.29** by in acidic or basic conditions, **4.29** was then ring opened leading to the triazole **4.30**. Due to the possible rotation, the triazole ring turned in order to obtain **4.31** which is in equilibrium with **4.32**. Finally, an attack of the nitrogen of the triazole to the previously formed aldehyde led to **4.33** and after aromatization gave the expected isomer triazolo[1,5-a]pyridine **4.34**.

Further studies have been done to confirm this rearrangement, in fact the NOE effect allowed us to determinate by NMR, the structure associated to each peak from LC/MS. Most of the triazolo[4,3-a]pyridine derivatives were converted into their corresponding Dimroth rearranged structures and we were to obtain simultaneously two libraries with triazolo[4,3-a]pyridine **4.24** and **4.27** and triazolo[1,5-a]pyridine **4.35** and **4.36**. Nevertheless, the scaffold containing a nitrogen linker on the triazole ring were

rearranged in lower yields, the additional nitrogen could have been involved in the increase of stability of the scaffold **4.27**.

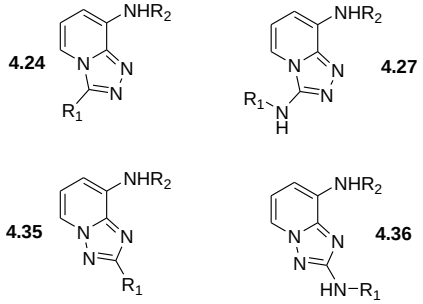
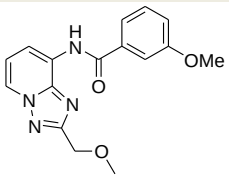
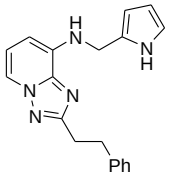
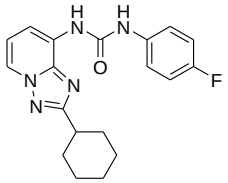
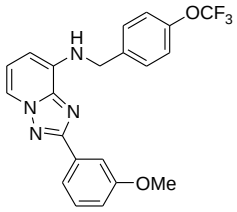
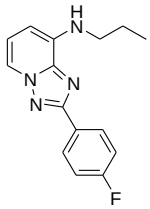
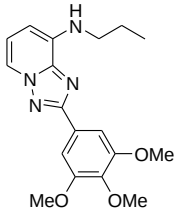
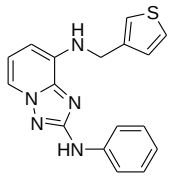
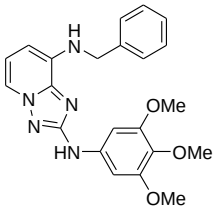


Figure 5. Triazolo[4,3-a]pyridine derivatives and triazolo[1,5-a]pyridine derivatives.

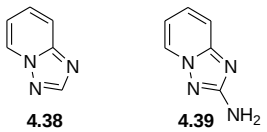
Table 5. Dimroth rearrangement: triazolo[1,5-a]pyridine derivatives **4.35** and **4.36**.

entry	starting material	triazolo[1,5-a]pyridine		yield (%)[a]
1	**4.24b**		**4.35a**	15
2	**4.24d**		**4.35b**	12
3	**4.24e**		**4.35c**	16
4	**4.24i**		**4.35d**	17
5	**4.24j**		**4.35e**	9
6	**4.24l**		**4.35f**	13

7	**4.27a**		**4.36a**	9
8	**4.27e**		**4.36b**	7

[a]Isolated yield.

The Dimroth rearrangement directed our efforts towards the synthesis of triazolo[1,5-a]pyridine aiming the evaluation of such chemistry for future projects. It was decided to use the 2-amino-4-methyl-pyridine **4.37** as model to assess this chemistry. Two possible pathways were available in literature[11] to synthesize the desired product, the first one leading to the scaffold not substituted on the triazole ring **4.38** and the second method allow the introduction of an amine group at the position 3 of the triazolo[1,5-a]pyridine **4.39**, both of these pathways have been attempted.



4.38 **4.39**

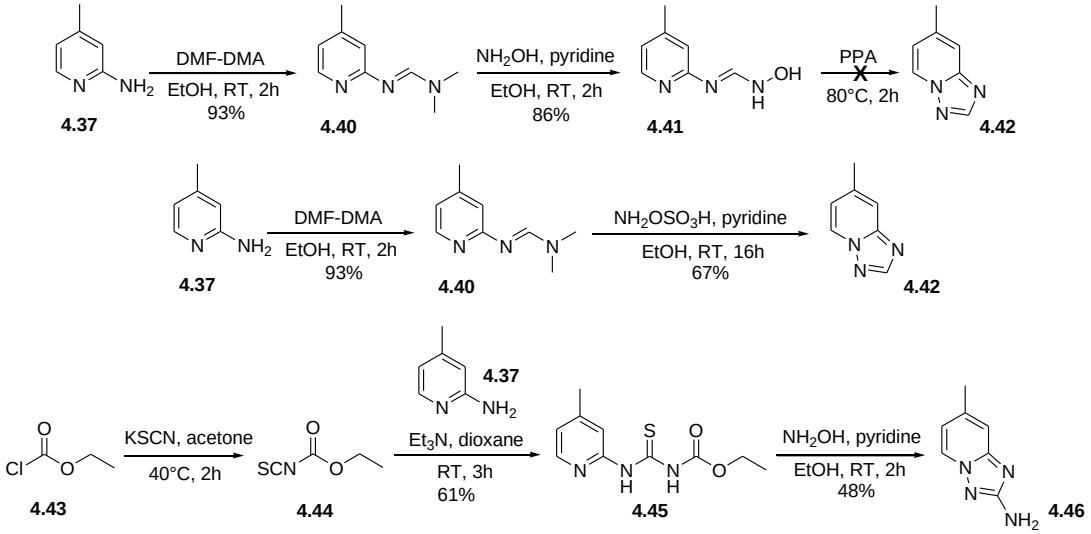
Figure 6. Triazolo[1,5-a]pyridine derivatives.

The synthesis started with the treatment of **4.37** in ethanol with DMF-DMA in order to obtain the intermediate **4.40** used directly without further purification, with hydroxylamine to lead to **4.41**. This late derivative was treated with a dehydrating reagent, the polyphosphoric acid at 80°C for 2 hours to obtain the desired scaffold **4.42** but the reaction with PPA degraded the starting material instead of to form a nitrene intermediate prior to final cyclization. To avoid this problem, we used a "self dehydrative reagent", the hydroxylamine-O-sulfonic acid, while this reagent was subjected to **4.41** in ethanol at room temperature for 16 hours, **4.42** was obtained in good yield.

The second part was dedicated to the synthesis of the scaffold containing an amine group, the same starting material **4.37** was used. 2-amino-4-methyl-pyridine reacted with the intermediate **4.44**, obtained from the reaction between ethyl chloroformate **4.43** and potassium thiocyanate in acetone, to afford the thiourea derivative **4.45**, which was

cyclized by treatment with hydroxylamine leading the 3-amino-7-methyl-triazolo[1,5-a]pyridine **4.46**.

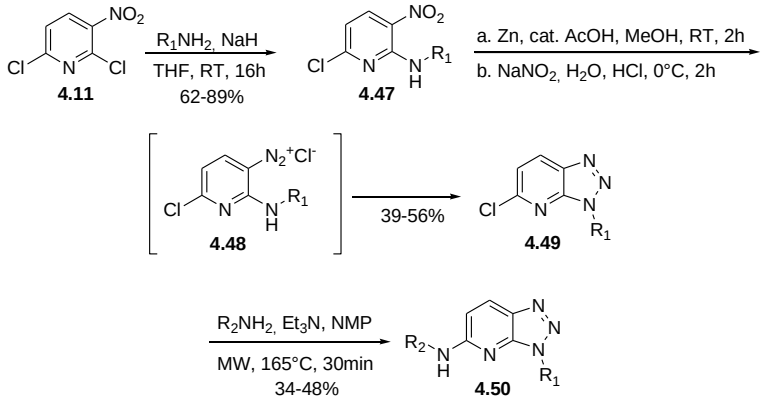
We were able to synthesize two analogues of triazolo[1,5-a]pyridine **4.42** and **4.46** in order to evaluate this chemistry and to make it available for future projects.



Scheme 10. Synthesis of triazolo[1,5-a]pyridine derivatives.

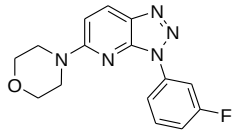
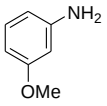
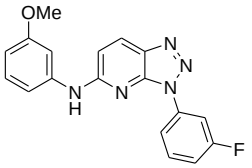
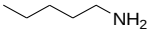
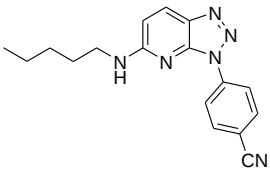
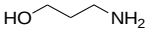
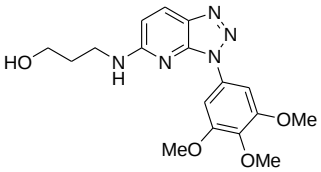
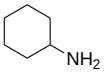
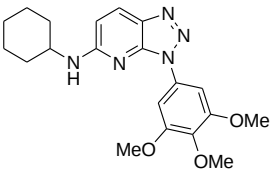
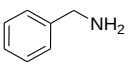
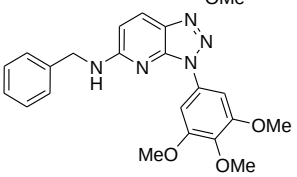
In order to extend the scope of this project, our interest led us to another triazolopyridine isomer, in fact the two previous examples involved the nitrogen of the pyridine during the cyclization. The triazolo[4,5-b]pyridine **4.3** as discussed in the introduction is structurally close to the purine, the fused ring involved in the kinase mechanisms.[12] The synthesis started with the nucleophilic substitution of the 2,6-dichloro-3-nitro pyridine **4.11** by an amine using sodium hydride as a base to afford the amino derivative **4.47**. The nitro group was then reduced by using zinc and a catalytic amount of acetic acid followed by a cyclization performed under Sandmeyer like conditions[13] to lead to the scaffold **4.49**, in fact the amino derivative was treated with sodium nitrite in water and hydrochloric acid at a tempertaure of 0°C for 2 hours. The use of the Sandmeyer like conditions led to the formation of a diazonium salt intermediate **4.48** which was attacked by the amine at the position 2 of the pyridine ring to afford the desired fused ring **4.49**. The last step of the pathway was the substitution of the chloride by an amine, due to the low reactivity of the chloro derivative **4.49** towards nucleophilic substitution, the reaction was performed using microwaves irradiation and high boiling solvent such as NMP. The intermediate **4.49** was microwaves irradiated

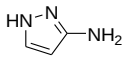
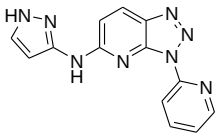
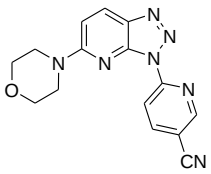
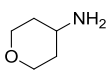
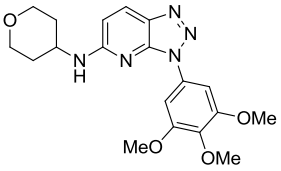
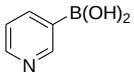
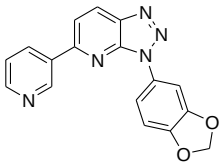
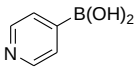
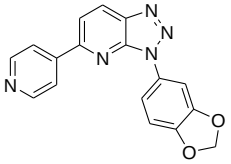
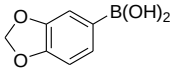
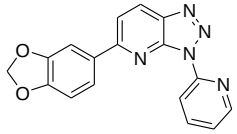
with an excess of various amines in N-methyl pyrrolidine at 165°C for 30 minutes to give the substituted triazolo[4,5-b]pyridine **4.50**.



Scheme 11. Synthesis of triazolo[4,5-b]pyridine derivatives **4.50**.

Table 6. Synthesis of triazolo[4,5-b]pyridine derivatives.[a]

entry	starting material	reagent	triazolo[4,5-b]pyridine		yield (%)[b]
1	**4.49a**			**4.50a**	48
2	**4.49a**			**4.50b**	37
3	**4.49b**			**4.50c**	41
4	**4.49c**			**4.50d**	35
5	**4.49c**			**4.50[e]**	48
6	**4.49c**			**4.50f**	44

7	**4.49d**			**4.50g**	34
8	**4.49e**			**4.50h**	45
9	**4.49f**			**4.50i**	43
10	**4.49g**			**4.51a**	51
11	**4.49g**			**4.51b**	49
12	**4.49h**			**4.51c**	53

[a]See the experimental section for details. [b]Isolated yield.

Three examples of Suzuki cross-coupling reaction have been synthesized from the derivative **4.49** and boronic acid leading to **4.51** with moderate yields. R_1 was generally an aryl or a heteroaryl group, R_2 was selected among aliphatic or cycloalkyl groups, nevertheless some analogues have been chosen as anilines in order to expand the library.

3. Biological evaluation

The libraries of the three isomers of triazolopyridine described in this chapter have been screened towards selected kinases of ongoing projects of Galapagos. The compounds were first tested in an enzymatic assay at constant concentration and for the analogues displaying an interesting percentage of inhibition for a specified kinase, a further assay was performed at different concentrations in order to obtain the half maximal inhibitory concentration IC_{50}.

Table 7. Biological evaluation of pyridazine derivatives (N/A not active).

entry	compounds	CDK9 (% inhibition at 10 μM)	IC_{50} (nM)	TGF-β II (% inhibition at 10 μM)	IC_{50} (nM)
1	**4.24a**	N/A	N/A	N/A	N/A
2	**4.24b**	N/A	N/A	N/A	N/A
3	**4.24c**	N/A	N/A	N/A	N/A
4	**4.24d**	N/A	N/A	N/A	N/A
5	**4.24**[e]	N/A	N/A	N/A	N/A
6	**4.24f**	13	N/A	8	N/A
7	**4.24g**	11	N/A	4	N/A
8	**4.24h**	22	N/A	10	N/A
9	**4.24i**	N/A	N/A	N/A	N/A
10	**4.24j**	21	N/A	N/A	N/A
11	**4.24k**	4	N/A	16	N/A
12	**4.24l**	4	N/A	N/A	N/A
13	**4.27a**	N/A	N/A	N/A	N/A
14	**4.27b**	98	N/A	27	N/A
15	**4.27d**	87	2800	49	N/A
16	**4.27i**	N/A	N/A	N/A	N/A
17	**4.27j**	N/A	N/A	N/A	N/A
18	**4.35a**	N/A	N/A	N/A	N/A
19	**4.35b**	N/A	N/A	N/A	N/A
20	**4.35c**	N/A	N/A	N/A	N/A
21	**4.35d**	N/A	N/A	N/A	N/A
22	**4.35**[e]	8	N/A	8	N/A
23	**4.35f**	12	N/A	8	N/A
24	**4.36a**	N/A	N/A	N/A	N/A
25	**4.36b**	16	N/A	5	N/A
26	**4.50a**	N/A	N/A	N/A	N/A
27	**4.50b**	N/A	N/A	N/A	N/A
28	**4.50c**	N/A	N/A	29	N/A
29	**4.50d**	2	N/A	35	N/A
30	**4.50**[e]	11	N/A	53	N/A

31	**4.50f**	3	N/A	27	N/A
32	**4.50g**	N/A	N/A	N/A	N/A
33	**4.50h**	N/A	N/A	N/A	N/A
34	**4.50i**	N/A	N/A	N/A	N/A
35	**4.51a**	47	N/A	99	600
36	**4.51b**	59	N/A	52	N/A
37	**4.51c**	35	N/A	36	N/A

entry	compounds	GSK3 (% inhibition at 20 μM)	IC_{50} (nM)	IRAK4 (% inhibition at 20 μM)	IC_{50} (nM)
38	**4.27c**	22	>20000	6	>20000
39	**4.27**[e]	38	>20000	13	>20000
40	**4.27f**	86	3644	65	>4000
41	**4.27g**	25	>20000	13	>20000
42	**4.27h**	28	>20000	8	>20000

The triazolo[4,3-a]pyridine has been the first scaffold screened towards CDK9 and TGF-β II receptor and only the 3-amino substituted derivative **4.27d** displayed a biological activity against the target (IC_{50}= 2800 nM), in fact only the analogues with a nitrogen linker at the position 3 of the triazolopyridine and an acylated group at the position 8 showed an interesting profile to develop such as **4.27f** (IC_{50}= 3644 nM towards GSK3). Most of the other congeners did not show a percentage of inhibition higher than 50 % and thus have not been screened at different concentrations. The analogues obtained from the Dimroth rearrangement leading to the triazolo[1,5-a]pyridine **4.35** and **4.36** have not displayed significant biological activity. The last scaffold assayed was the triazolo[4,5-b]pyridine **4.50** and **4.51**, the 5-amino substituted analogues did not give activity towards the kinases targeted but the compound **4.51a** without linker at the position 5 obtained from a palladium cross-coupling displayed a half maximal inhibitory concentration IC_{50}= 600 nM towards TGF-β II receptor.

4. Conclusion

This chapter dealt with the synthesis of three different isomers of triazolopyridine, despite the fact that this collection corresponds to the largest library of derivatives explored during this PhD, only a partial part has been discussed.

In a first part, a study concerning the synthesis of triazolo[4,3-a]pyridine derivatives with two points of substitution at the positions 5 and 8 has been envisioned, several problems appeared during the attempts of cyclization with trialkyl orthoformate. By using formic acid hydrazide, it was possible to obtain the desired scaffold, two examples of final product have been synthesized but due to the low overall yield of the process and the difficulty to reproduce the experiments, we decided to change the pattern of substitution of the triazolo[4,3-a]pyridine. In fact, the modification of the decoration position to the triazole ring was made by using acid chloride derivatives with the 2-hydrazino-3-nitro-pyridine intermediate followed by a treatment with phosphorus oxychloride to afford the scaffold substituted at the position 3. It was possible to obtain a (hetero)aryl group directly attached to the triazolo[4,3-a]pyridine with the use of acid chloride, in order to cover others analogues, isocyanate and isothiocyanate reagents have been used to introduce a nitrogen linker between the (hetero)aryl group and the scaffold, Various examples have been synthesized to build a library of triazolo[4,3-a]pyridine.

The second part discussed was related to the partial conversion of the initial scaffold triazolo[4,3-a]pyridine into triazolo[1,5-a]pyridine by Dimroth rearrangement under acidic or basic conditions. The presence of the electron withdrawing nitro group promotes this conversion but the separation of the two isomers was possible and the NOE effect allowed us to determinate which structures correspond to the desired scaffold and to the other isomer. Due to this rearrangement and in order to prepare pathways for future projects, the synthesis of triazolo[1,5-a]pyridine has been studied and we were able to obtain two examples of such scaffold by using 2-amino-4-methyl-pyridine as a model.

The last part of this chapter dealt with the development of a library of triazolo[4,5-b]pyridine derivatives starting from 2,6-dichloro-3-nitro-pyridine and using a cyclization based on Sandmeyer like conditions through a diazonium salt intermediate.

5. Experimental section

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 ºC) under a nitrogen or argon atmosphere. Reaction temperatures are reported as bath temperature. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Compounds were visualized with UV light (λ = 254 nm). Products were purified by flash chromatography on ICN silica gel 63-200, 60 Å. Melting points were obtained on a melting point apparatus Electrothermal IA9200 with open capillary tubes. ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz, 500 MHz and 600 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as the solvent. The ^{1}H and ^{13}C chemical shifts were referenced to residual solvent signals at δ H/C 7.26/77.00 ($CDCl_3$), 3.31/49.10 and 2.50/39.50 (DMSO-d_6) relative to TMS as internal standard. Coupling constants *J* [Hz] were directly taken from the spectra. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High resolution mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Electrospray MS spectra were obtained on a Micromass platform LC/MS spectrometer. Column used for all LC/MS analysis: Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm ID x 50 mm L. All the methods are using MeCN/H_2O gradients. Water contains either 0.1 % TFA or 0.1 % NH_3.

6-Chloro-2-hydrazinyl-3-nitropyridine (4.12)

To a solution of 2,6-dichloro-3-nitropyridine **4.11** (1 g, 5.2 mmol) in 20 mL of EtOH under argon at room temperature was added a solution of hydrazine hydrate 64% (252 μL, 5.2 mmol). The reaction mixture was stirred for 2 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The filtrate was concentrated to afford the desired compound **4.12** in 62% yield as orange solid. Data for **4.12**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89-8.98 (m, 1H), 8.47 (d, *J* = 7.8 Hz, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 6.53-6.64 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.3, 155.0, 136.7, 128.2, 110.4.

5-Chloro-8-nitro-triazolo[4,3-a]pyridine (4.13)

To a solution of 2,6-dichloro-3-nitropyridine **4.11** (1 g, 5.2 mmol) in 20 mL of dioxane under argon at room temperature was added formic acid hydrazide (312 mg, 5.2 mmol). The reaction mixture was stirred for 2 hours at room temperature. After which $POCl_3$ (634 μL, 6.8 mmol) was added and the reaction refluxed for 16 hours. The completion of the reaction was monitored by TLC. The solvent was concentrated in vacuo and 30 mL of saturated aqueous $NaHCO_3$ was added to the residue, then extracted with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/MeOH = 10:1) to afford the desired compound **4.13** in 26% yield as orange solid. Data for

4.13: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.32 (d, *J* = 10.2 Hz, 1H), 5.94 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.2, 136.5, 127.1, 118.9, 113.0, 109.1.

General procedure for the preparation of triazolo[4,3-a]pyridine derivatives 4.16:

To a solution of **4.13** (199 mg, 1.0 mmol) in 5 mL of dioxane under argon was added an amine (1.1 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by flash column chromatography on silica gel (EtOAc) to afford the amino substituted triazolo[4,3-a]pyridine **4.16**.

N-ethyl-8-nitro-triazolo[4,3-a]pyridin-5-amine (4.16a)

Brown solid, 35% yield; Data for **4.16a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.31 (d, *J* = 9.2 Hz, 1H), 6.24 (m, 1H), 5.74 (d, *J* = 9.5 Hz, 1H), 3.37 (m, 2H), 1.16 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.4, 149.0, 137.3, 132.3, 128.6, 121.8, 36.5, 14.9.

4-(8-Nitro-triazolo[4,3-a]pyridin-5-yl)morpholine (4.16b)

Brown solid, 24% yield; Data for **4.16b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.24 (d, *J* = 9.5 Hz, 1H), 5.55 (d, *J* = 9.6 Hz, 1H), 3.78-3.81 (m, 4H), 3.12-3.15 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.0, 148.1, 138.6, 135.3, 128.2, 119.9, 65.7, 48.4.

N-(5-(ethylamino)-triazolo[4,3-a]pyridin-8-yl)acetamide (4.17a)

To a solution of **4.16a** (207 mg, 1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of CH_2Cl_2 under argon was added an acetyl chloride (86 μL, 1.2 mmol) followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted triazolo[4,3-a]pyridine **4.17a** in 7% yield as brown solid. Data for **4.17a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (br. s, NH), 8.42 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 6.93-6.95 (t, *J* = 5.8 Hz, 1H), 6.14 (d, *J* = 8.5 Hz, 1H), 2.09 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.9, 152.2, 145.9, 140.8, 124.4, 114.2, 88.7, 36.8, 23.7, 14.3.

N-benzyl-5-morpholino-triazolo[4,3-a]pyridin-8-amine (4.17b)

To a solution of **4.16b** (249 mg, 1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of EtOH under argon was added benzaldehyde (111 μL, 1.1 mmol) followed by sodium cyanoborohydride (126 mg, 2.0 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the amino substituted triazolo[4,3-a]pyridine **4.17b** in 10% yield as brown solid. Data for **4.17b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (d, *J* = 4.8 Hz, 1H), 7.66 (s, 1H), 7.41-7.48 (m, 2H), 7.27-7.41 (m, 3H), 5.99 (d, *J* = 4.8 Hz, 1H), 4.41 (s, 2H), 3.93-4.01 (m, 4H), 366-3.74 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.8, 145.5, 139.0, 138.8, 131.9, 128.2, 127.5, 126.9, 120.8, 91.2, 66.0, 51.7, 47.9.

5-Chloro-3-methyl-8-nitro-triazolo[4,3-a]pyridine (4.18)

To a solution of **4.12** (189 mg, 1.0 mmol) in 20 mL of dioxane under argon at room temperature was added acetyl chloride (79 μL, 1.1 mmol) followed by triethylamine (181 μL, 1.3 mmol). The reaction mixture was stirred for 2 hours at room temperature. After which $POCl_3$ (121 μL, 1.3 mmol) was added and the reaction refluxed for 16 hours. The completion of the reaction was monitored by TLC. The solvent was concentrated in vacuo and 30 mL of saturated aqueous $NaHCO_3$ was added to the residue, then extracted with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/MeOH = 10:1) to afford the desired compound **4.18** in 35% yield as orange solid. Data for **4.18**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (d, *J* = 9.9 Hz, 1H), 5.85 (d, *J* = 9.9 Hz, 1H), 2.80 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.9, 136.4, 128.1, 126.9, 111.7, 15.5.

2-Hydrazinyl-3-nitropyridine (4.21)

To a solution of 2-chloro-3-nitropyridine **4.20** (5 g, 31.5 mmol) in 20 mL of EtOH under argon at room temperature was added a solution of hydrazine hydrate 64% (3.0 mL, 63.0 mmol). The reaction mixture was stirred for 2 hours. The suspension formed was filtered, then washed several times with EtOH, and dried to yield **4.21** in 93% yield as yellow solid. Data for **4.21**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01-9.67 (m, 1H), 8.48-8.52 (m, 1H), 8.34-8.40 (m, 1H), 6.92-7.57 (m, 2H), 6.70-6.76 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.2, 152.1, 135.4, 126.6, 111.8.

General procedure for the preparation of triazolo[4,3-a]pyridine derivatives 4.23:

To a solution of **4.21** (154 mg, 1 mmol) in 20 mL of dioxane under argon at room temperature was added an acid chloride (1.1 mmol) followed by triethylamine (181 μL, 1.3 mmol). The reaction mixture was stirred for 2 hours at room temperature. After which $POCl_3$ (121 μL, 1.3 mmol) was added and the reaction refluxed for 16 hours. The completion of the reaction was monitored by TLC. The suspension formed was filtered, then washed several times with EtOH, and dried to afford **4.23**.

3-(Methoxymethyl)-8-nitro-triazolo[4,3-a]pyridine (4.23a)

Brown solid, 77% yield; Data for **4.23a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98-9.13 (m, 1H), 8.67 (d, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.3 Hz, 1H), 5.07 (s, 2H), 3.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 145.7, 142.3, 135.1, 132.0, 130.1, 113.6, 63.1, 58.1.

3-cyclohexyl-8-nitro-[1,2,4]triazolo[4,3-a]pyridine (4.23c)

Brown solid, 71% yield; Data for **4.23c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (d, *J* = 6.3 Hz, 1H), 8.68 (d, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.0 Hz, 1H), 3.38-3.47 (m, 1H), 2.00-2.09 (m, 2H), 1.30-1.89 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.5, 141.0, 134.9, 131.8, 130.3, 113.4, 33.0, 30.3, 25.4, 25.3.

3-(methoxymethyl)-N-(pyridin-3-ylmethyl)-triazolo[4,3-a]pyridin-8-amine (4.24a)

Brown solid, 29% yield; Data for **4.24a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62-8.65 (m, 1H), 8.43-8.46 (m, 1H), 7.77-7.80 (m, 1H), 7.66 (d, *J* = 6.7 Hz, 1H), 7.31-7.37 (m, 2H), 6.74 (t, *J* = 7.1 Hz, 1H), 6.09 (d, *J* = 7.4 Hz, 1H), 4.89 (s, 2H), 4.54 (d, *J* = 6.4 Hz, 2H), 3.29 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 148.9, 148.3, 144.6, 135.1, 135.0, 134.8, 123.6, 115.5, 111.3, 98.3, 63.4, 57.7, 43.4.

3-Methoxy-N-(3-(methoxymethyl)-triazolo[4,3-a]pyridin-8-yl)benzamide (4.24b)

Brown solid, 31% yield; Data for **4.24b**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.37 (br. s, NH), 8.28 (d, *J* = 6.8 Hz, 1H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.57-7.58 (m, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.20-7.22 (m, 1H), 7.08 (t, *J* = 7.1 Hz, 1H), 4.99 (s, 2H), 3.86 (s, 3H), 3.32 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.9, 159.4, 145.9, 145.0, 135.2, 129.9, 125.8, 120.2, 120.1, 118.2, 117.3, 114.3, 113.0, 63.3, 57.8, 55.5.

N-((1H-pyrrol-2-yl)methyl)-3-phenethyl-triazolo[4,3-a]pyridin-8-amine (4.24d)

Beige solid, 14% yield; Data for **4.24d**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.70 (br. s, NH), 7.62 (d, *J* = 6.7 Hz, 1H), 7.17-7.30 (m, 5H), 6.64-6.73 (m, 2H), 6.48 (t, *J* = 5.9 Hz, 1H), 6.17 (d, *J* = 7.3 Hz, 1H), 5.98-6.01 (m, 1H), 5.90-5.93 (m, 1H), 4.38 (d, *J* = 6.0 Hz, 2H), 3.28-3.32 (m, 2H), 3.09-3.15 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.9, 144.5, 140.8, 135.4, 128.7, 128.6, 128.4, 126.3, 117.3, 115.0, 110.7, 107., 106.4, 97.6, 32.3, 25.9.

1-(3-Cyclohexyl-triazolo[4,3-a]pyridin-8-yl)-3-(4-fluorophenyl)urea (4.24e)

White solid, 11% yield; Data for **4.24e**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.61 (br. s, NH), 9.24 (br. s, NH), 8.11 (d, *J* = 6.6 Hz, 1H), 7.88 (d, *J* = 6.9 Hz, 1H), 7.47-7.50 (m, 2H), 7.14-7.17 (m, 2H), 6.90 (t, *J* = 7.1 Hz, 1H), 3.20-3.26 (m, 1H), 1.99-2.04 (m, 2H), 1.80-1.85 (m, 2H), 1.62-1.76 (m, 3H), 1.44-1.51 (m, 2H), 1.30-1.36 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 158.5, 156.9, 152.4, 151.8, 144.0, 135.6, 127.2, 120.0, 116.3, 115.7, 115.5, 114.2, 108.4, 33.4, 30.4, 25.6, 25.5.

3-(3-Methoxyphenyl)-N-(4-(trifluoromethoxy)benzyl)-triazolo[4,3-a]pyridin-8-amine (4.24i)

Orange solid, 22% yield; Data for **4.24i**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.78 (d, *J* = 6.8 Hz, 1H), 7.51-7.54 (m, 3H), 7.46 (t, *J* = 6.4 Hz, 1H), 7.41-7.43 (m, 1H), 7.37-7.38 (m, 1H), 7.32 (d, *J* = 8.3 Hz, 2H), 7.13-7.15 (m, 1H), 6.75 (t, *J* = 7.1 Hz, 1H), 6.04 (d, *J* = 7.4 Hz, 1H), 4.56 (d, *J* = 6.4 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.8, 147.3, 146.8, 145.2, 138.9, 135.4, 130.5, 128.9, 128.3, 121.1, 120.2, 116.2, 115.9, 113.2, 110.8, 98.2, 55.4, 44.9.

3-(4-Fluorophenyl)-N-propyl-triazolo[4,3-a]pyridin-8-amine (4.24j)

Yellow solid, 28% yield; Data for **4.24j**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90-7.96 (m, 2H), 7.74 (d, *J* = 6.7 Hz, 1H), 7.42-7.48 (m, 2H), 6.83 (t, *J* = 7.1 Hz, 1H), 6.57 (t, *J* = 5.9 Hz, 1H), 6.17 (d, *J* = 7.4 Hz, 1H), 3.23 (q, *J* = 6.3 Hz, 2H), 1.61-1.73 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 161.3, 146.1, 145.2, 136.1, 130.6, 123.7, 116.6, 116.3, 110.0, 97.1, 44.2, 21.4, 11.6.

General procedure for the preparation of triazolo[4,3-a]pyridine derivatives 4.26:

To a solution of **4.21** (154 mg, 1 mmol) in 20 mL of dioxane under argon at room temperature was added an isocyanate or isothiocyanate (1.1 mmol). The reaction mixture was stirred for 2 hours at room temperature. After which $POCl_3$ (121 μL, 1.3 mmol) was added and the reaction refluxed for 16 hours. The completion of the reaction was monitored by TLC. The suspension formed was filtered, then washed several times with EtOH, and dried to afford **4.26**.

N-(4-fluorophenyl)-8-nitro-triazolo[4,3-a]pyridin-3-amine (4.26b)

Beige solid, 61% yield; Data for **4.26b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (br. s, NH), 9.23 (d, *J* = 6.7 Hz, 1H), 8.65 (d, *J* = 7.2 Hz, 1H), 7.89-7.96 (m, 2H), 7.11 (t, *J* = 6.8 Hz, 1H), 6.54-6.59 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.9, 155.6, 144.2, 136.0, 135.7, 123.1, 121.4, 118.3, 116.0, 113.8.

8-Nitro-N-(pyridin-2-yl)-triazolo[4,3-a]pyridin-3-amine (4.26d)

Brown solid, 55% yield; Data for **4.26d**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.61 (br. s, NH), 9.26 (d, *J* = 6.4 Hz, 1H), 8.59 (d, *J* = 7.2 Hz, 1H), 8.29-8.31 (m, 1H), 8.09-8.12 (m, 1H), 7.71-7.85 (m, 2H), 7.22-7.27 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.7, 148.2, 147.5, 138.7, 138.2, 134.7, 130.9, 128.8, 117.2, 111.1, 110.9.

N3-(4-fluorophenyl)-N8-isobutyl-triazolo[4,3-a]pyridine-3,8-diamine (4.27c)

Yellow solid, 21% yield; Data for **4.27c**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (br. s, NH), 7.96 (d, *J* = 6.0 Hz, 1H), 7.68-7.73 (m, 2H), 7.11 (t, *J* = 8.9 Hz, 2H), 6.77-6.84 (m, 1H), 6.44-6.46 (m, 1H), 5.67 (t, *J* = 6.0 Hz, 1H), 3.08 (t, *J* = 6.5 Hz, 2H), 1.93-2.03 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.7, 157.9, 142.6, 138.3, 135.5, 117.7, 115.5, 115.1, 113.5, 102.3, 50.3, 27.3, 20.4.

N-(3-((4-fluorophenyl)amino)-triazolo[4,3-a]pyridin-8-yl)butyramide (4.27d)

Beige solid, 37% yield; Data for **4.27d**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (d, *J* = 7.9 Hz, 1H), 8.12 (d, *J* = 6.4 Hz, 1H), 8.09 (br. s, NH), 7.52-7.54 (m, 2H), 7.39 (br. s, NH), 7.03-7.05 (m, 2H), 6.86-6.88 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 1.73-1.79 (m, 2H), 1.00 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.1, 161.2, 158.7, 157.1, 143.4, 136.1, 125.0, 122.2, 118.7, 116.3, 115.7, 112.7, 39.4, 18.8, 13.7.

N-(3-((3,4,5-trimethoxyphenyl)amino)-triazolo[4,3-a]pyridin-8-yl)cyclohexanecarboxamide (4.27f)

Brown solid, 12% yield; Data for **4.27f**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.62 (br. s, NH), 9.42 (br. s, NH), 8.48-8.50 (m, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.14 (s, 2H), 6.95-6.99 (m, 1H), 3.80 (s, 6H), 3.62 (s, 3H), 2.55-2.66 (m, 1H), 1.61-1.92 (m, 5H), 1.17-1.49 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.2, 161.6, 153.1, 144.5, 137.7, 131.5, 124.8, 123.4, 118.8, 112.2, 95.0, 60.3, 55.8, 44.3, 29.4, 25.5, 25.3.

N-(3-((3,4,5-trimethoxyphenyl)amino)-triazolo[4,3-a]pyridin-8-yl)butyramide (4.27g)

Brown solid, 13% yield; Data for **4.27g**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.78 (br. s, NH), 9.41 (br. s, NH), 8.49 (d, *J* = 6.4 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.13 (s, 2H), 6.97 (t, *J* = 6.7 Hz, 1H), 3.79 (s, 6H), 3.61 (s, 3H), 2.45 (t, *J* = 7.2 Hz, 2H), 1.61-1.66 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.3, 161.5, 153.0, 144.4, 137.6, 131.4, 124.7, 123.5, 118.9, 112.1, 94.9, 60.3, 55.8, 38.0, 18.7, 13.7.

N8-benzyl-N3-(4-fluorophenyl)-triazolo[4,3-a]pyridine-3,8-diamine (4.27j)

Beige solid, 10% yield; Data for **4.27j**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.13 (br. s, NH), 7.57-7.60 (m, 3H), 7.39 (d, *J* = 7.3 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.15 (t, *J* = 8.9 Hz, 2H), 7.09 (t, *J* = 6.2 Hz, 1H), 6.62 (t, *J* = 7.1 Hz, 1H), 5.85 (d, *J* = 7.3 Hz, 1H), 4.48 (d, *J* = 6.2 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.4, 155.8, 145.4, 141.7, 139.4, 138.1, 135.4, 128.5, 127.1, 126.9, 117.7, 115.5, 114.1, 109.6, 96.6, 45.8.

3-Methoxy-N-(2-(methoxymethyl)-triazolo[1,5-a]pyridin-8-yl)benzamide (4.35a)

Brown solid, 15% yield; Data for **4.35a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.37 (br. s, NH), 8.77 (d, *J* = 6.8 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.57-7.64 (m, 2H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.19-7.27 (m, 2H), 4.64 (s, 2H), 3.86 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7, 162.3, 159.3, 146.7, 135.2, 129.8, 126.7, 125.2, 121.3, 120.1, 118.1, 114.2, 113.1, 67.1, 58.1, 55.5.

2-(3-Methoxyphenyl)-N-(4-(trifluoromethoxy)benzyl)-triazolo[1,5-a]pyridin-8-amine (4.35d)

Orange solid, 17% yield; Data for **4.35d**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (d, *J* = 7.1 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.72-7.73 (m, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.19 (t, *J* = 6.3 Hz, 1H), 7.05-7.07 (m, 1H), 6.88 (t, *J* = 7.2 Hz, 1H), 6.33 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 6.2 Hz, 2H), 3.84 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.4, 159.9, 147.6, 144.9, 139.2, 136.7, 132.6, 130.4, 129.2, 121.4, 119.4, 116.6, 116.2, 115.9, 111.8, 102.7, 55.6, 45.4.

N-propyl-2-(3,4,5-trimethoxyphenyl)-triazolo[1,5-a]pyridin-8-amine (4.35f)

Brown solid, 13% yield; Data for **4.35f**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (d, *J* = 6.2 Hz, 1H), 7.48 (s, 2H), 6.95 (t, *J* = 7.6 Hz, 1H), 6.46 (d, *J* = 7.7 Hz, 1H), 6.36 (t, *J* = 5.7 Hz, NH), 3.89 (s, 6H), 3.74 (s, 3H), 3.22 (q, *J* = 6.2 Hz, 2H), 1.61-1.75 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 153.2, 144.6, 138.9, 137.0, 126.5, 115.7, 115.5, 103.8, 101.4, 60.2, 56.0, 44.3, 21.4, 11.6.

7-Methyl-triazolo[1,5-a]pyridine (4.42)

To a solution of **4.37** (108 mg, 1.0 mmol) in 10 mL of EtOH under argon at room temperature was added DMF-DMA (201 μL, 1.5 mmol). The reaction mixture was stirred for 2 hours at room temperature. After completion of the reaction monitored by TLC, 20 mL of water was added to quench the excess of dimethyl formamide-dimethyl acetal, an extraction with 2x15 mL of CH_2Cl_2 was performed, and the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The orange oil was directly used for the next step without further purification.

To a solution of **4.40** in 10 mL of EtOH under argon at room temperature was added hydroxylamine-O-sulfonic acid (170 mg, 1.5 mmol) followed by pyridine (121 μL, 1.5 mmol). The reaction mixture was stirred for 16 hours at room temperature. After completion of the reaction monitored by TLC, 30 mL of saturated aqueous $NaHCO_3$ was added to the residue, then extracted with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/MeOH = 8:1) to afford the desired compound **4.42** in 67% yield as white solid. Data for **4.42**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (d, *J* = 6.8 Hz, 1H), 8.39 (s, 1H), 7.60 (br. s, 1H), 7.02 (dd, *J* = 6.8, 1.6 Hz, 1H), 2.42 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 154.2, 150.5, 141.5, 128.4, 116.9, 114.9, 21.2.

7-Methyl-triazolo[1,5-a]pyridin-2-amine (4.46)

To a solution of **4.43** (109 mg, 1.0 mmol) in 20 mL of acetone under argon at room temperature was added potassium thiocyanate (117 mg, 1.2 mmol). The reaction mixture was heated at 40 °C for 2 hours. After which the formation of a precipitate has been observed (NaCl), then 20 mL of water was added and an extraction with 2x15 mL of CH_2Cl_2 was performed, and the organic layer was dried over Na_2SO_4 and concentrated in controlled vacuo (**4.44** has a low boiling point). The yellow liquid was directly used for the next step without further purification.

To a solution of **4.44** in 10 mL of dioxane under argon at room temperature was added **4.37** (108 mg, 1.0 mmol) followed by triethylamine (181 μL, 1.3 mmol). The reaction mixture was stirred for 3 hours at room temperature. After completion of the reaction monitored by TLC, 30 mL of saturated aqueous $NaHCO_3$ was added to the residue, then extracted with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The pink oil was directly used for the next step without further purification.

To a solution of **4.45** in 10 mL of EtOH under argon at room temperature was added hydroxylamine hydrochloride (104 mg, 1.5 mmol) followed by pyridine (121 μL, 1.5 mmol). The reaction mixture was stirred for 2 hours at room temperature. After completion of the reaction monitored by TLC, 30 mL of saturated aqueous $NaHCO_3$ was added to the residue, then extracted with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (EtOAc/MeOH = 8:1) to afford the desired compound **4.46** in 48% yield as pale yellow solid. Data for **4.46**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (d, *J* = 6.8 Hz, 1H), 7.14 (s, 1H), 6.68-6.69 (m, 1H), 5.86 (br. s, NH_2), 2.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.9, 145.2, 139.0, 127.1, 116.9, 115.8, 20.6.

General procedure for the preparation of pyridine derivatives 4.47:

To a solution of **4.11** (193 mg, 1.0 mmol) in 20 mL of THF under argon was added sodium hydride (60%) (44 mg, 1.1 mmol), followed after 30 minutes by an amine (1.1 mmol) at room temperature. The reaction mixture was stirred until the completion of the reaction (16 hours monitored by TLC) and was allowed to cool to room temperature. 50 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x20 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to afford the amino substituted pyridine **4.47**.

General procedure for the preparation of triazolo[4,5-b]pyridine derivatives 4.49:

To a solution of **4.47** (1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 2 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of HCl was added dropwise a solution of sodium nitrite (104 mg, 1.5 mmol) in 5 mL of water at 0 °C. The reaction mixture was stirred for 2 hours, after which 50 mL of water was added to the reaction mixture followed by a portionwise addition of K_2CO_3 until basic pH, an extraction with 2x20 mL of AcOEt was performed. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc/MeOH = 9:1) to afford the desired triazolo[4,5-b]pyridine derivatives **4.49**.

General procedure for the preparation of triazolo[4,5-b]pyridine derivatives 4.50:

To a solution of **4.49** (1.0 mmol) in 3 mL of NMP was added an amine (1.5 mmol), followed by triethylamine (181 μL, 1.3 mmol) at room temperature. The reaction mixture was irradiated by microwaves at 165 °C for 30minutes and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the triazolo[4,5-b]pyridine analogues **4.50**.

4-(3-(3-Fluorophenyl)-3H-triazolo[4,5-b]pyridin-5-yl)morpholine (4.50a)

White solid, 48% yield; Data for **4.50a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (d, *J* = 9.4 Hz, 1H), 8.08-8.17 (m, 2H), 7.66-7.74 (m, 1H), 7.29-7.37 (m, 1H), 7.16 (d, *J* = 9.4 Hz, 1H), 3.71-3.77 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.3, 131.8, 131.7, 130.0, 116.3, 114.5, 114.2, 107.8, 107.4, 66.0, 45.4.

3-(3-Fluorophenyl)-N-(3-methoxyphenyl)-3H-triazolo[4,5-b]pyridin-5-amine (4.50b)

Brown solid, 37% yield; Data for **4.50b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.97 (br. s, NH), 8.30-8.34 (m, 1H), 8.09-8.17 (m, 2H), 7.61-7.75 (m, 2H), 7.33-7.42 (m, 1H), 7.24-7.27 (m, 2H), 7.01-7.05 (m, 1H), 6.61-6.65 (m, 1H), 3.74 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.9, 156.4, 141.4, 132.4, 131.6, 129.6, 116.9, 114.9, 114.6, 112.4, 111.7, 108.3, 104.8, 55.1.

6-(5-morpholino-3H-triazolo[4,5-b]pyridin-3-yl)nicotinonitrile (4.50h)

Beige solid, 45% yield; Data for **4.50h**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.15-9.16 (m, 1H), 8.761-8.66 (m, 1H), 8.55-8.58 (m, 1H), 8.34 (d, *J* = 9.4 Hz, 1H), 7.17 (d, *J* = 9.3 Hz, 1H), 3.72-3.77 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.7, 152.9, 143.3, 130.1, 127.6, 122.8, 115.4, 107.7, 65.9, 45.3.

N-(tetrahydro-2H-pyran-4-yl)-3-(3,4,5-trimethoxyphenyl)-3H-triazolo[4,5-b]pyridin-5-amine (4.50i)

Brown solid, 43% yield; Data for **4.50i**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (d, *J* = 9.1 Hz, 1H), 7.72 (d, *J* = 7.0 Hz, 1H), 7.66 (s, 2H), 6.70 (d, *J* = 9.1 Hz, 1H), 4.06-4.17 (m, 1H), 3.87-3.95 (m, 8H), 3.74 (s, 3H), 3.36-3.43 (m, 2H), 1.95-2.04 (m, 2H), 1.45-1.59 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.6, 153.4, 145.0, 132.8, 131.6, 128.9, 97.8, 66.1, 60.4, 56.1, 46.8, 32.3.

3-(Benzo[d][1,3]dioxol-5-yl)-5-(pyridin-4-yl)-3H-triazolo[4,5-b]pyridine (4.51b)

Beige solid, 49% yield; Data for **4.51b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.74-8.89 (m, 3H), 8.32 (d, *J* = 8.8 Hz, 1H), 8.17-8.21 (m, 2H), 7.73-7.81 (m, 2H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.22 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 150.8, 148.2, 137.5, 130.5, 121.7, 118.8, 116.2, 109.9, 108.9, 103.8, 102.3.

6. References

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Chapter V. General discussion

Protein kinases have long been recognized as important drug targets, there are more than 500 kinases in the human genome, a large number of putative kinase inhibitors are in clinical studies and they are present in the portfolios of most of the pharmaceutical companies.

In fact, many of them are known to play a critical role in biological systems and will become potential drug targets for various human diseases including cancer, cardiovascular disorders, and inflammation in the future. The main approach to inhibit a kinase is to block the ATP-binding, the vast majority of kinase inhibitors make at least one hydrogen bond interaction with the hinge region.

Selectivity seems to be a major issue and it was feared that use of such kinase directed cores would lead to unselective compounds. However, various examples are described where selectivity is obtained by choosing the right decoration on a particular scaffold that has itself interactions with the hinge region.

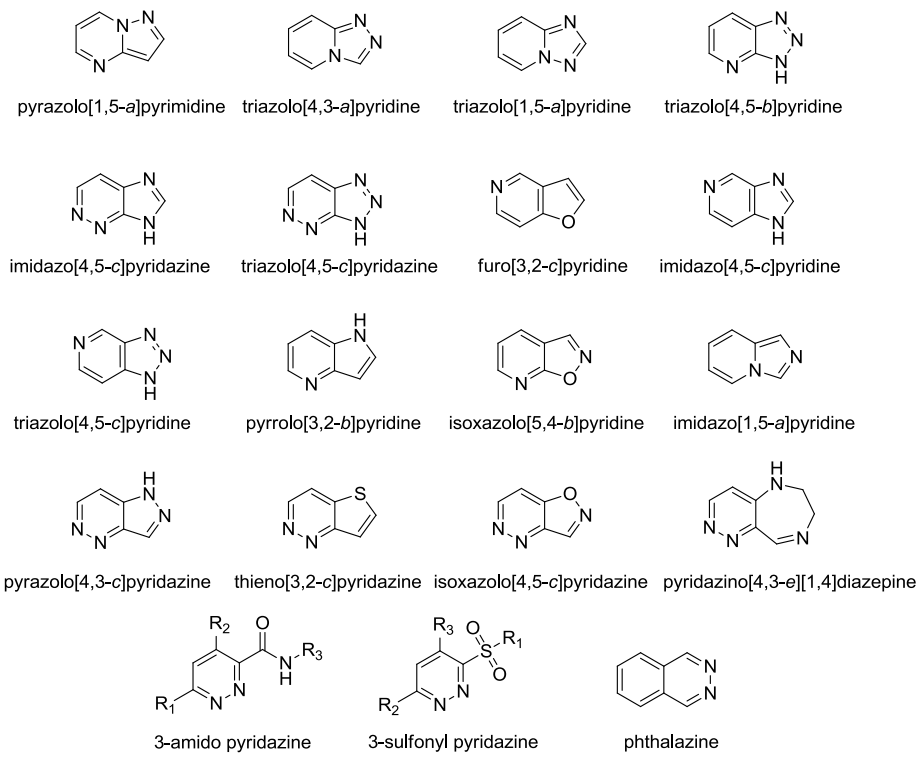
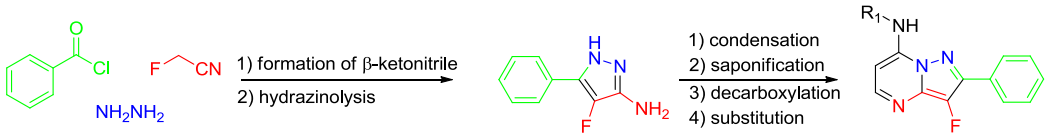


Figure 1. List of the scaffolds synthesized during the PhD.

This PhD research was devoted to the design and synthesis of new compound libraries targeting kinases. The elaboration of 19 scaffolds has been performed and more

than 250 analogues have been synthesized and screened towards different kinases of ongoing projects from Galapagos NV.

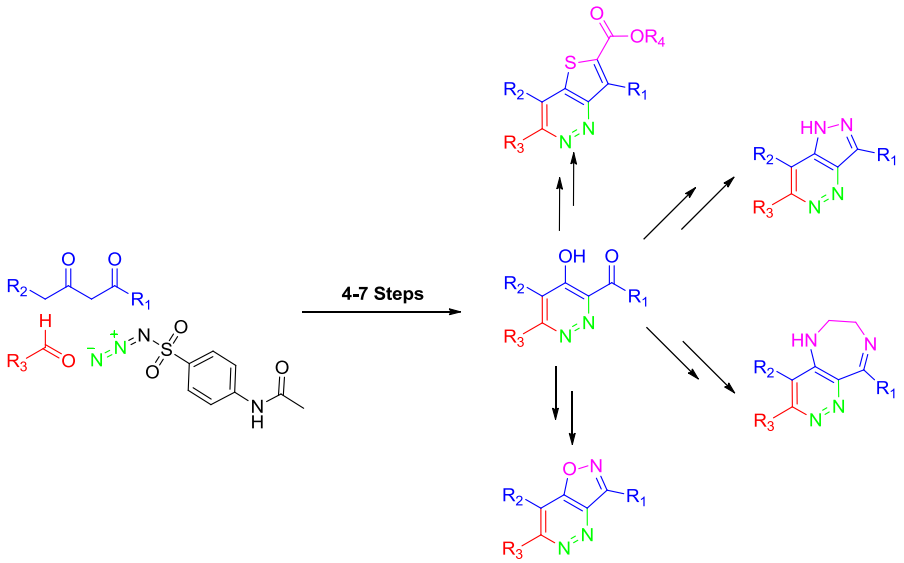
In the chapter II, the research has been focused on the synthesis of pyrazolo[1,5-a]pyrimidine and libraries associated, after different attempts of synthesis, the method chosen has been the one described by Gavrin et al. Two points of decoration has been selected and various linkers have been applied to a large number of derivatives. In order to evaluate fluorinated analogues, a simple approach has been elaborated and applied to the synthesis of 3-fluoro pyrazolo[1,5-a]pyrimidine. A 37-member has been screened towards ongoing kinase projects of Galapagos and the compound **2.29b** gave an IC_{50} of 859 nM towards MAP4K4.



R_1= aliphatic amine, cycloalkyl amine, benzyl amine and heteroaryl amine

Scheme 1. Simple approach to the synthesis of 3-fluoro pyrazolo[1,5-a]pyrimidine.

In the chapter III, an emphasis has been done on the pyridazine ring and related fused rings. In fact, a number of methodology has been developed aiming to access particular 1,2-diazines with different functional groups in order to build a library. A first route has been elaborated to methyl 6-substituted 4-hydroxypyridazine carboxylate from the readily available methyl acetoacetate allowing a large number of substitutions.

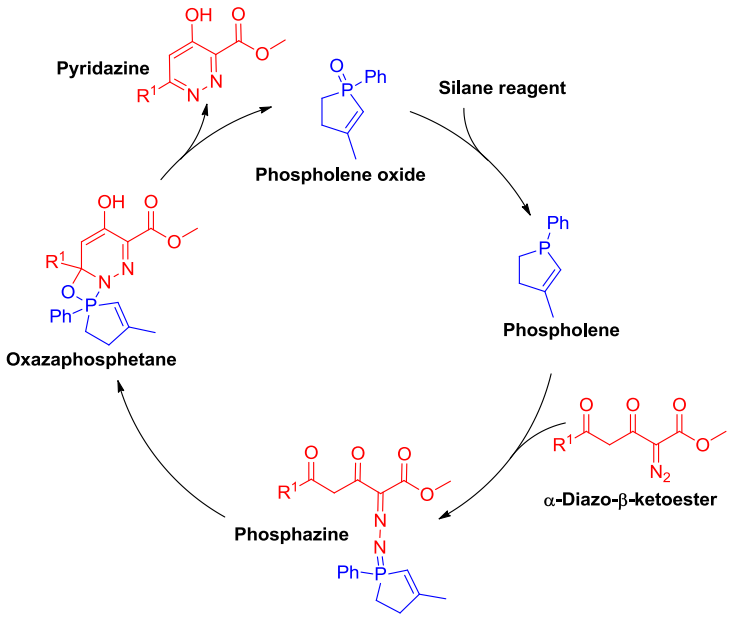


Scheme 2. Strategy for the synthesis of pyridazine heterocycles and their derivatives.

This strategy has been optimized with the replacement of the toxic HMPT by P(*n*-Bu)$_3$, this modification led to a reduction of the reaction time to 15 minutes instead of 16 hours and to obtain the pyridazines as precipitate without further purification.

Based on this methodology, it was possible to synthesize unknown heterocycles such as 5H,6H,7H-pyridazino[4,3-e][1,4]diazepine or isoxazolo[4,5-c]pyridazine analogues.

We were then concerned about the underexplored Diaza-Wittig reaction, in fact we believed that such reaction needed a catalytic alternative and inspired by the work performed by numerous groups, we developed the first catalytic Diaza-Wittig applied to the synthesis of pyridazines and a novel catalytic Aza-Wittig reaction applied to the synthesis of 1,4-benzodiazepines.



Scheme 3. Catalytic Diaza-Wittig reaction.

A library of 44 analogues has been developed and screened towards kinase, the best derivative **3.29d** displayed a half maximal inhibitory concentration IC_{50} = 290 nM.

In the chapter IV, we discussed about three isomers of the triazolopyridine scaffold. Our first interest was the synthesis of a library of triazolo[4,3-a]pyridine analogues. After a short evaluation of the pattern of substitution, it was decided to have two point of attachments on the pyridine ring and on the triazole ring. The introduction of

substitution was made possible by selecting the appropriate acid chloride or iso(thio)cyanate.

We observed the formation of an isomer during the LC/MS control, after NMR study and literature review, the isomer was identified as the triazolo[1,5-a]pyridine obtained from the Dimroth rearrangement enhanced by the presence of a nitro group. We explored routes for the synthesis of such derivatives and screened the isomers obtained.

The last isomer studied was the triazolo[4,5-b]pyridine, which do not involve the nitrogen from the pyridine in the fused cyclization, the key step of the synthesis was the formation of the triazole ring by the generation of a diazonium salt intermediate under "Sandmeyer-like" conditions.

A 42-member library has been screened towards kinase by Galapagos and we obtained one derivative **4.51a** with an IC_{50}= 600 nM towards TGF-β II receptor.

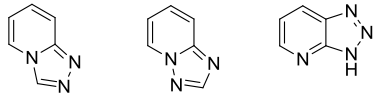
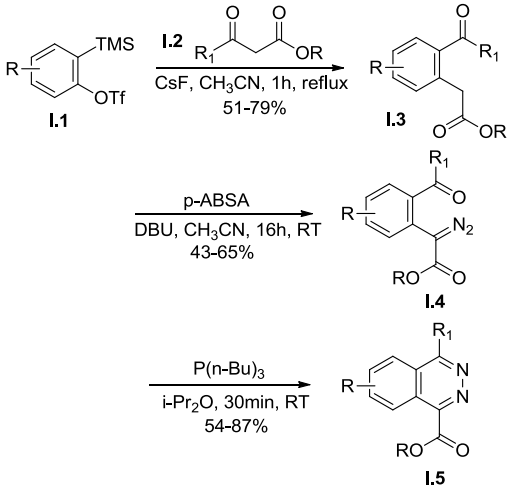
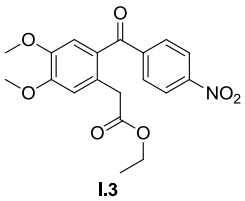


Figure 2. Triazolopyridine isomers.

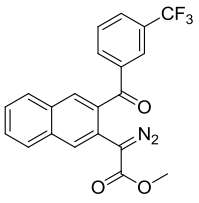
Appendix I: Synthesis of Phthalazine derivatives



Scheme 1. Synthesis of phthalazine derivatives from an aryne precursor.

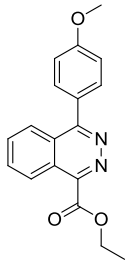


Representative procedure for **I.3**: To a solution of ethyl 3-(4-nitrophenyl)-3-oxopropanoate **I.2** (261 mg, 1.1 mmol) and 4,5-dimethoxy-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **I.1** (500 mg, 1.4 mmol) in 15 mL of CH_3CN under argon was added anhydrous Cesium fluoride (425 mg, 2.8 mmol) at room temperature. The reaction mixture was refluxed for 1 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled down to room temperature, and a saturated solution of NaCl (15 mL) was added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and purified by flash chromatography (hexane/EtOAc 4:1) to provide the ethyl 2-[4,5-dimethoxy-2-(4-nitrobenzoyl)phenyl]acetate **I.3** as a yellow oil (227 mg, 55%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 6.87 (s, 1H), 6.86 (s, 1H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.97 (s, 3H), 3.89 (s, 2H), 3.77 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.9, 171.0, 151.5, 149.6, 146.8, 143.4, 130.6, 128.9, 128.6, 123.1, 114.6, 113.8, 60.6, 55.9, 55.8, 38.5, 13.8.



I.4

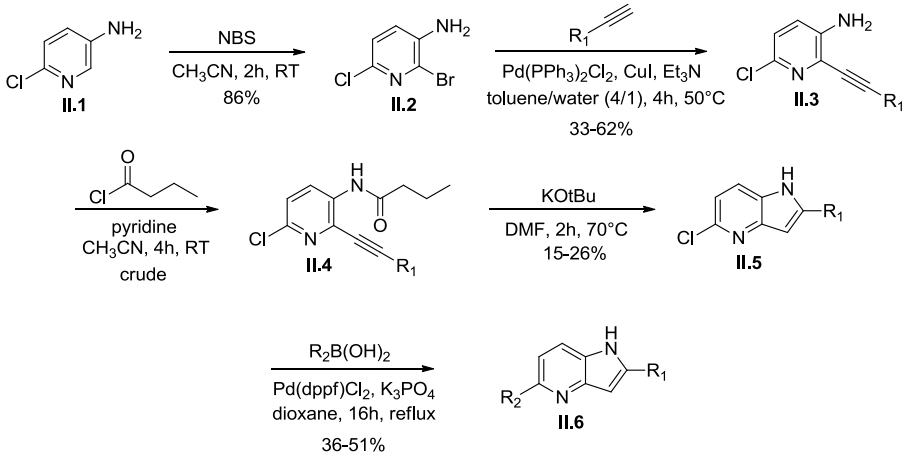
Representative procedure for **I.4**: To a solution of acyl-alkyl derivative **I.3** (372 mg, 1 mmol) in 10 mL of CH_3CN under argon at 0 °C was added *p*-acetamido benzene sulfonyl azide (*p*-ABSA) (240 mg, 1 mmol) followed by DBU (194 μL, 1.3 mmol). The mixture was stirred for 16 h and after completion of the reaction (monitored by TLC), a saturated solution of NH_4Cl (15 mL) was added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and purified by flash chromatography (hexane/EtOAc 3:1) to provide the methyl 2-diazo-2-{3-[3-(trifluoromethyl)benzoyl]naphthalen-2-yl}acetate **I.4** as an orange oil (203 mg, 51%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (s, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 8.00 (s, 1H), 7.87-7.94 (m, 4H), 7.57-7.68 (m, 3H), 3.64 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 194.9, 166.1, 138.3, 134.8, 134.4, 133.5, 131.7, 131.4, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 127.9, 127.8, 127.0, 126.9, 122.1, 52.2. Diazo carbon was not detected in ^{13}C NMR.



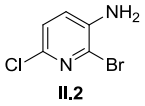
I.5

Representative procedure for **I.5**: To a solution of diazo compound **I.4** (324 mg, 1 mmol) in 10 mL of *i*-Pr_2O was added P(*n*-Bu)$_3$ (250 μL, 1 mmol) at room temperature. The reaction mixture was stirred at room temperature for 30 minutes and a yellow suspension has been formed. The suspension was filtered, washed with *i*-Pr_2O and dried to afford the ethyl 4-(4-methoxyphenyl)phthalazine-1-carboxylate **I.5** as a pale yellow solid (182 mg, 59%); mp 126-129°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49 (d, *J* = 8.2 Hz, 1H), 8.10-8.14 (m, 3H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 4.57 (q, *J* = 7.0 Hz, 2H), 3.90 (s, 3H), 1.44 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.0, 160.7, 160.6, 149.7, 133.7, 131.8, 127.8, 126.7, 125.0, 124.1, 114.3, 62.4, 55.5, 14.2. HRMS calcd for $C_{18}H_{16}N_2O_3$ $(M+H)^+$: 309.1234, found: 309.1237.

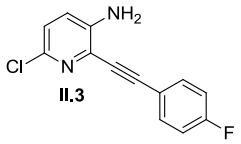
Appendix II: Synthesis of 4-azaindole derivatives



Scheme 2. Synthesis of 4-azaindole derivatives.

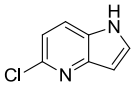


Representative procedure for **II.2**: To a solution of 2-Chloro-5-aminopyridine **II.1** (129 mg, 1.0 mmol) in 15 mL of CH_3CN under argon was added NBS (214 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred for 2 h. After completion of the reaction (monitored by TLC), a saturated solution of $NaHCO_3$ (30 mL) as well as CH_2Cl_2 were added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to provide the 2-Bromo-6-chloropyridin-3-amine **II.2** as a brown solid in 86% yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.30 (d, *J* = 8.3 Hz, 1H), 7.09 (d, *J* = 8.3 Hz, 1H), 5.79 (br. s, NH2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 143.1, 133.9, 124.9, 124.7, 124.2.



Representative procedure for **II.3**: To a solution of 2-Bromo-6-chloropyridin-3-amine **II.2** (208 mg, 1.0 mmol) in 12 mL of toluene and 3 mL of water under argon was added 1-ethynyl-4-fluorobenzene (138 µL, 1.2 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol), CuI (19 mg, 0.1 mmol) and triethylamine (306 µL, 2.2 mmol) at room temperature. The reaction mixture was stirred for 4 h at 50°C. After completion of the reaction (monitored by TLC), a saturated solution of $NaHCO_3$ (30 mL) as well as CH_2Cl_2 were added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and purified by flash chromatography (hexane/EtOAc 3:1) to provide the 6-chloro-2-((4-fluorophenyl)ethynyl)pyridin-3-amine **II.3** as a pale yellow solid in 53% yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.69-7.74 (m, 2H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 8.6 Hz, 1H), 7.23-7.27 (m, 2H),

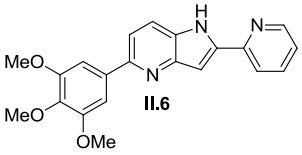
5.55 (br. s, NH2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.7, 145.3, 142.1, 129.8, 127.3, 123.9, 117.8, 116.0, 98.4, 67.6.



II.5

Representative procedure for **II.5**: To a solution of 6-chloro-2-((trimethylsilyl)ethynyl)pyridin-3-amine **II.3** (225 mg, 1.0 mmol) in 15 mL of CH_2Cl_2 under argon was added butyryl chloride (136 μL, 1.3 mmol) followed by pyridine (105 mg, 1.3 mmol) at 0°C. The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction (monitored by TLC), a saturated solution of $NaHCO_3$ (30 mL) was added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and directly used for the next step without further purification.

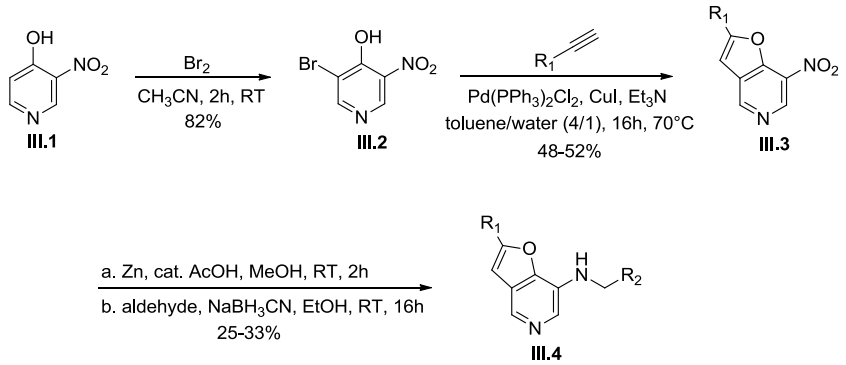
To a solution of the previously prepared crude **II.4** in 10 mL of DMF was added portionwise KOtBu (281 mg, 2.5 mmol), the reaction mixture was heated at 70°C for 2 h and after completion of the reaction (monitored by TLC), a saturated solution of $NaHCO_3$ (30 mL) and CH_2Cl_2 were added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to provide the 5-chloro-pyrrolo[3,2-b]pyridine **II.5** as a white solid 15% in yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.56 (br. s, NH), 7.84 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 3.2 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 6.54 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.7, 142.5, 130.8, 127.3, 122.0, 115.9, 101.3.



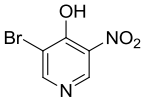
II.6

Representative procedure for **II.6**: To a solution of 5-chloro-2-(pyridin-2-yl)-1H-pyrrolo[3,2-b]pyridine **II.5** (230 mg, 1.0 mmol) in 5 mL of dioxane under argon was added (3,4,5-trimethoxyphenyl)boronic acid (254 mg, 1.2 mmol), K_3PO_4 (467 mg, 2.2 mmol) and 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium (82 mg, 0.1 mmol) at room temperature. The reaction mixture was refluxed until the completion of the reaction for 16 hours and then was allowed to cool to room temperature. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the 2-(pyridin-2-yl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrrolo[3,2-b]pyridine **II.6** as a brown solid in 42% yield; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.93 (br. s, NH), 8.68 (d, *J* = 4.2 Hz, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.93 (t, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.38-7.42 (m, 4H), 3.90 (s, 6H), 3.72 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 153.1, 149.9, 149.8, 149.4, 146.4, 140.5, 137.8, 137.3, 131.5, 129.4, 123.1, 120.8, 119.7, 15.1, 103.8, 101.1, 60.2, 56.0.

Appendix III: Synthesis of furo[3,2-c]pyridine derivatives

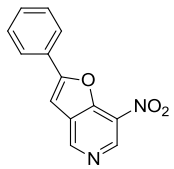


Scheme 2. Synthesis of furo[3,2-c]pyridine derivatives.



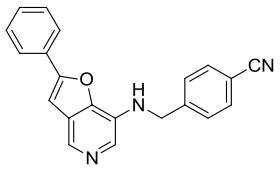
III.2

Representative procedure for **III.2**: To a solution of 4-hydroxy-3-nitropyridine **III.1** (140 mg, 1.0 mmol) in 10 mL of water was slowly added bromine (67 µL, 1.3 mmol) at room temperature. The reaction mixture was heated for 2 h at 50°C and then cooled to room temperature. The resulting precipitate was filtered, washed with water, and then dried to give the 3-bromo-5-nitropyridin-4-ol **III.2** as a white solid in 82% yield. The product was directly used without further purification for the next step; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.76 (br. s, OH), 8.83 (s, 1H), 8.37 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.7, 139.2, 138.9, 137.1, 118.8.



III.3

Representative procedure for **III.3**: To a solution of 3-bromo-5-nitropyridin-4-ol **III.2** (219 mg, 1.0 mmol) in 12 mL of toluene and 3 mL of water under argon was added phenylacetylene (132 µL, 1.2 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol), CuI (19 mg, 0.1 mmol) and triethylamine (306 µL, 2.2 mmol) at room temperature. The reaction mixture was stirred for 16 h at 70°C. After completion of the reaction (monitored by TLC), a saturated solution of $NaHCO_3$ (30 mL) as well as CH_2Cl_2 were added. The phases were separated and the aqueous layer was extracted two times with CH_2Cl_2 (15 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and purified by flash chromatography (hexane/EtOAc 2:1) to provide the 7-nitro-2-phenylfuro[3,2-c]pyridine **III.3** as a beige solid in 52% yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 9.17 (s, 1H), 7.97 (d, J = 7.8 Hz, 2H), 7.74 (s, 1H), 7.49-7.60 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.6, 150.9, 148.0, 140.1, 130.1, 129.5, 128.8, 128.2, 127.7, 125.4, 98.3.



III.4

Representative procedure for **III.4**: To a solution of **III.3** (240 mg, 1.0 mmol) in 5 mL of MeOH under argon was added a catalytic amount of acetic acid (6 μL, 0.1 mmol) followed by zinc (327 mg, 5.0 mmol) at room temperature. The reaction mixture was stirred for 5 hours. 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The reduced derivative was directly used for the next step without further purification.

To a solution of the amine (1.0 mmol) in 5 mL of EtOH under argon was added 4-cyanobenzaldehyde (170 mg, 1.3 mmol) followed by sodium cyanoborohydride (126 mg, 2.0 mmol) at room temperature. The reaction mixture was stirred for 16 hours, after which 15 mL of saturated aqueous $NaHCO_3$ was added to the reaction mixture followed by an extraction with 2x15 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The product was purified by preparative HPLC to afford the 4-(((2-phenylfuro[3,2-c]pyridin-7-yl)amino)methyl)benzonitrile **III.4** as a white solid in 31% yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.98 (d, *J* = 7.2 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.72 (s, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.54 (t, *J* = 7.1 Hz, 2H), 7.42-7.47 (m, 2H), 6.93 (t, *J* = 6.3 Hz, NH), 4.67 (d, *J* = 6.3 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.4, 146.7, 146.3, 132.4, 132.3, 130.2, 129.4, 129.3, 129.1, 128.1, 127.4, 126.0, 124.9, 119.0, 109.6, 100.2, 46.1.

CV and publications

Bel Abed Hassen was born in 1985 in Grasse, France. He started to study chemistry at the University of Marseille, France where he obtained his B. Sc. in Chemistry in 2006 and then moved to the University of Montpellier, France where he obtained his M. Sc. in Chemistry in 2008. He also worked as a Synthetic Organic Chemist for Charabot, Robertet, L'Oréal and Syngenta before to start in September 2009 an industrial PhD project at the Rega Institute for Medical Research, KU Leuven in collaboration with the Galapagos company under the supervision of Prof. Dr. Piet Herdewijn and Dr. Guy Van Lommen.

Bel Abed, H.; Mammoliti, O.; Van Lommen, G.; Herdewijn, P. "A short and convenient strategy for the synthesis of pyridazines via Diaza-Wittig reaction." *Tetrahedron Letters* **2012**, *53*, 6489-6491.

Bel Abed, H.; Mammoliti, O.; Van Lommen, G.; Herdewijn, P. "Simple approach to the synthesis of 3-fluoro pyrazolo[1,5-a]pyrimidine analogues." *Tetrahedron Letters* **2013**, *54*, 2612-2614.

Bel Abed, H.; Mammoliti, O.; Bande, O.; Van Lommen, G.; Herdewijn, P. "Strategy for the synthesis of pyridazine heterocycles and their derivatives." *Journal of Organic Chemistry* **2013**, *78*, 7845-7858.

Bel Abed, H.; Bande, O.; Mammoliti, O.; Van Lommen, G.; Herdewijn, P. "A novel and convenient strategy for the synthesis of phthalazines from an aryne precursor." *Tetrahedron Letters* **2013**, manuscript accepted.